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## Systematics of the Northern Andean *Isoëtes karstenii* Complex

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**ABSTRACT.**—The *Isoëtes karstenii* complex includes those species characterized by laevigate megaspores, acute to free ala apices, a highly reduced labium, and distributions in the high altitude páramos of Venezuela, Colombia, and Ecuador. These species have been variously treated in the literature. Many early authors placed all Andean laevigate-spored specimens in *Isoëtes lechleri*, a central Andean species, while one later author recognized up to eight northern Andean species distinct from *I. lechleri*. This research represents a revisionary study to determine the number and identity of the taxa in this complex. Multivariate and descriptive analyses of morphological data and chromosome counts were performed. Five species are recognized including the previously described *I. karstenii* and *I. palmeri*. One variety is transferred from *I. lechleri* to *I. karstenii* (*I. karstenii* var. *anomala*). Three of the recognized species are newly described: *I. fuliginosa*, *I. hemivelata* and *I. precocia*. Chromosome counts revealed that *I. karstenii* and *I. precocia* are diploids ( $2n = 22$ ) and that *I. palmeri* is tetraploid ( $2n = 44$ ). Estimates of chromosome number based on spore size for *I. fuliginosa* and *I. hemivelata* indicate that they are polyploid ( $2n \geq 44$ ).

The laevigate-spored taxa of *Isoëtes* L. from the northern Andes have long presented taxonomists with considerable difficulty. These difficulties are due largely to the overall morphological similarity of the species and the wide range of morphological variation within the species. Also, few collections of these species were available for study until the field work of A.M. Cleef and collaborators and J. and S. Keeley in the 1970s and 1980s. As part of continuing efforts to describe and characterize the Neotropical species of *Isoëtes* we undertook a revisionary study of this difficult group of laevigate-spored species, the *Isoëtes karstenii* A. Braun complex (Hickey, 1985).

The goals of this study were to: (1) determine the number of taxa that should be included within the *I. karstenii* complex, (2) clarify the taxonomy of the species, and (3) document intra- and interspecific morphological variation. To accomplish these goals we have collected morphological data and conducted principal components analyses of those data to infer morphologically discontinuous groups. In addition we have determined or estimated chromosome numbers for the included species. Finally, we present a taxonomic treatment that formalizes our understanding of the delimitations and relationships among these species.

### TAXONOMIC HISTORY OF THE *ISOËTES KARSTENII* COMPLEX

The *Isoëtes karstenii* complex was first delimited by Hickey (1985) in a morphologically based cladistic analysis of Neotropical *Isoëtes*. It includes those



TABLE 1. Summary of the taxonomic recognition of the species historically associated with the *Isoëtes karstenii* complex. A “+” indicates the taxon was accepted as valid by the author(s); an “S” that the taxon was considered a synonym of *I. lechleri*; a “V” that the taxon was considered a variety of *I. lechleri*.

	<i>Isoëtes lechleri</i>	<i>Isoëtes karstenii</i>	<i>Isoëtes socia</i>	<i>Isoëtes colombi- ana</i>	<i>Isoëtes rimbachi- ana</i>	<i>Isoëtes palmeri</i>	<i>Isoëtes cleefii</i>
Mettenius (1859)	+						
Braun (1862)	+	+	+				
Baker (1880)	+	S	S				
Motelay & Vendryes (1882)	+	S	S				
Underwood (1888)	+	S	S				
Pfeiffer (1922)	+	S	S				
Weber (1922)	+	+	S				
Plamer (1929, 1932)	+	S	S	V	V		
Rodriguez (1955)	+	S	S		V		
Vareschi (1968)	+	V			V		
Gomez (1980)	+	S	S	S	S		
Fuchs-Eckert (1982)	+	+	+	+	+	+	+
Hickey (1985)	+	+	+		+	+	+

taxa with laevigate megaspores, truncate to free ala apices and corneous (darkly sclerified) leaf apices. The complex is distributed throughout the páramos of Colombia and Venezuela at elevations above 3000 m, with a single disjunct location at Mt. Chimborazo, Ecuador. Hickey (1985) included five species in this complex: *Isoëtes cleefii* H. P. Fuchs, *Isoëtes palmeri* H. P. Fuchs, *Isoëtes karstenii* A. Braun, *Isoëtes socia* A. Braun (sensu Fuchs-Eckert, 1982), and *Isoëtes rimbachiana* H. P. Fuchs. In a compendium of South American species, Fuchs-Eckert (1982) recognized eight laevigate-spored species for Venezuela, Colombia, and Ecuador and therefore may be associated with the *I. karstenii* complex: *I. socia*, *I. karstenii*, *Isoëtes steyermarkii* (nom. nud.), *I. palmeri*, *I. cleefii*, *I. rimbachiana*, *I. colombiana* (T. C. Palmer) H. P. Fuchs, and *Isoëtes glacialis* Asplund (including *Isoëtes arumiana* [nom. nud.] pro syn.). Fuchs-Eckert (1982), however, did not recognize these species as a distinct group. Historically these names have been treated in the literature as distinct species, or more often, as synonyms or varieties of *Isoëtes lechleri* Mett., a central Andean (southern Peru and Bolivia) species (Table 1).

MATERIALS AND METHODS

MORPHOLOGY.—Approximately 200 herbarium sheets representing about 90 discrete collection numbers of Venezuelan, Colombian, and Ecuadorian laevigate-spored *Isoëtes* were examined. We gratefully acknowledge the following herbaria for use of material: B, BM, COL, F, G, GH, MO, MU, NY, U, UC, US and VEN. Sixty-three of the collections were chosen and scored for eight quantitative morphological characters; those collections that were excluded from the analyses consisted of immature or poorly preserved material. Characters



used in the ordination and statistical analyses were: leaf number, leaf length (mm), leaf width (mm) at the mid-length, ala length/leaf length ratio, length (mm) of corneous portion of leaf apex, velum coverage (scored as a discrete character; see below), megaspore diameter ( $\mu\text{m}$ ), and microspore length ( $\mu\text{m}$ ). Additional characters were scored for a subset of the collections but were abandoned due to non-discriminatory age-specific correlation (e.g., corm dimensions and sporangial dimensions) or extreme variation within individuals and collections (e.g., sporangial shape and ala apex morphology). Some characters used in the analyses are also correlated with age (e.g., leaf number), or showed variability within collections (e.g., leaf length). Despite this, these characters also appeared to show group specific and discriminatory ranges of variation and were therefore retained. Terminology for morphological characters follows Hickey (1985), and for two-dimensional shapes Radford et al. (1972).

Velum coverage was scored as: (1) complete to nearly complete ( $> 90\%$ ), or as (2) less than half covered (25–50%). For ordination analyses, velum coverage was therefore scored as a discrete character, complete vs. incomplete.

Although microspore length and width were measured, only microspore length was chosen for the ordination and statistical analyses because most previous workers have used microspore length, rather than width, some reporting only length values. Correlation between these two characters is high for 55 collections analyzed ( $r^2 = 0.877$ ), and either character should adequately represent the collection.

Measurements were taken only from mature plants and mature organs of those plants. Each collection, regardless of number of sheets or individuals, was considered representative of a single population unless evidence of hybridization (e.g., aborted spores) or multiple taxa within a collection (e.g., gross discontinuities in morphology) were present. Several individuals per collection were scored for each character (with the exception of microspore dimensions which were taken from a single sporangium) and a mean score for each character was calculated. The number of measurements per character differed from collection to collection. Some collections consist of only a single plant on a single herbarium sheet while others consist of numerous individuals on numerous sheets. In addition, the quality of materials varied greatly: many specimens were partially destroyed by insects, many were pressed with the substrate around the corm, and many were pressed in large clumps. As a result, some characters were difficult to obtain for all collections. Ten measurements per character per collection (25 for megaspores and microspores) were recorded whenever possible.

Two types of analyses of morphological data were conducted: (1) principal component analysis (PCA) and (2) character by character scatter plots. These analyses were performed in an attempt to reveal morphological discontinuities between groups of collections and were conducted without assigning collections to specific taxa, except for type specimens which were so designated. This was done to determine, without bias, whether or not distinct clusters of collections would be revealed which could be designated as putative morphological species and to determine which type specimen(s) would be associated



with which cluster. Once morphologically cohesive groups were recognized, descriptive statistics were calculated to document putative intraspecific variation. Multivariate and statistical analyses were performed using NTSYS version 1.80 (Rohlf, 1993) and Statmost v. 2.50 (DataMost).

**CYTOLOGY.**—Methods and materials for chromosome counts generally follow those of Hickey (1984). Voucher specimens for the materials from which counts were made are deposited at LOC, MU, and BM (Table 2). All specimens from which root tips were collected were grown completely submerged and potted in either fine quartz sand or a 3:1 mixture of sphagnum peat: fine quartz sand (Taylor and Luebke, 1986). Cells with mitotic figures were best obtained in root tips collected in the late morning (11:00 a.m.  $\pm$  one hour). Young roots about 1 cm long were harvested and immediately placed in a saturated paradichlorobenzene solution, incubated at room temperature for 4–5 hr, fixed in Farmer's solution (3:1 95% ethanol: glacial acetic acid) and refrigerated until squashed. Root tips were hydrolyzed in 1N hydrochloric acid at 60°C for 20–30 min, neutralized in a 95% ethanol series, stained in Whittman's hematoxylin for 25 min at room temperature, rinsed in glacial acetic acid to destain, and squashed in Hoyer's mounting media. Squash slides were examined and photographed at 600 $\times$  or 1000 $\times$ . Color photocopies of the photographic slides were then prepared. These photocopies were placed on a light box, covered with a plain piece of white paper and the chromosomes traced onto the white paper from which counts were made. Simultaneous viewing of the photographic and/or microscope slides and the photocopy aided in interpreting slides in which chromosomes overlapped.

When live material was unavailable, we attempted to estimate chromosome number by plotting mean megaspore diameter vs. mean microspore length for taxa of known chromosome number and then plotting the mean value of spore sizes of the unknown taxa onto that plot. A strong correlation exists between spore size and chromosome number in *Isoëtes* (Cox and Hickey, 1984; Britton and Goltz, 1991). For northeastern North American taxa, Kott and Britton (1983) noted that "ploidy level can be determined with some certainty from spore measurements, even for dried herbarium material." Mean values for spore sizes of 27 taxa of known chromosome number were taken from Rury (1978), Kott and Britton (1983), Britton and Goltz (1991), Brunton et al. (1994), Taylor et al. (1993), Hickey (1994), Musselman and Knepper (1994), Brunton and Britton (1996a, 1996b), and Watanabe et al. (1996). While such correlations are unlikely to definitively identify the chromosome number of an unknown species, they may be useful in obtaining an estimate, or as in our case (see below), in ruling out potential ploidy levels.

## RESULTS

**MORPHOLOGY.**—Included among the collections examined were type specimens (holo-, iso-, and/or paratypes) of *Isoëtes arumiana* (nom. nud.), *I. cleefii*, *I. colombiana*, *I. glacialis*, *I. karstenii*, *I. lechleri*, *I. palmeri*, *I. rimbachiana*, *I.*



TABLE 2. Chromosome counts, collection location, and voucher specimen data.

	Count	Collection Location	Voucher Location
<i>I. karstenii</i>	2n = 22	Pond, 8 km N of Pico de Aquila, Mérida, Venezuela	Small 4 & Hickey (MU)
	2n = 22	Pond, 15 km N of Pico de Aquila, Mérida, Venezuela	Small 5 & Hickey (MU)
	2n = 22	Pond, 15 km N of pico de Aquila, Mérida, Venezuela	Small 6 & Hickey (MU)
	2n = 22	Laguna Negra, Mérida, Venezuela	Small 7 & Hickey (MU)
	2n = 22	Laguna Mucubaji, Mérida, Venezuela	Small 8 & Hickey (MU)
	2n = 22	Pond, Paramo de Pisva, Municipio de Socota, Boyaca, Colombia	Small 128 & Gonzalez (MU, COL)
	2n = 22	Laguna La Pintada; Sierra Nevada del Cocuy, Boyaca, Colombia	Small 141-143 & Gonzalez (MU, COL)
	2n = 22	Laguna La Cuadrada, Sierra Nevada del Cocuy, Boyaca, Colombia	Small 144 & Gonzalez (MU, COL)
	2n = 22	Small pool near Laguna Chisaca, Cundinamarca, Colombia	Jermy 17479 (BM)
	2n = 44	Small pool near Laguna Chisaca, Cundinamarca, Colombia	Small 155-157 & Gonzalez (MU, COL)
<i>I. palmeri</i>	2n = 44	Small pool near Laguna Chisaca, Cundinamarca, Colombia	Jermy 17477 (BM)
	2n = 22	Small pool near Laguna Chisaca, Cundinamarca, Colombia	Jermy 17482 (BM)
<i>I. precocia</i>	2n = 22	Small pool S of Laguna Larga, Cundinamarca, Colombia	Keeley & Keeley 11032 (LOC)



TABLE 3. Eigenvectors for the first three principal components (PC) from the PCA.

Character	PC 1	PC 2	PC 3
Leaf length	0.21957	0.77593	0.21528
Leaf width	0.63580	0.16820	-0.61498
Microspore length	0.84544	-0.13050	0.28320
Megaspore diameter	0.76647	-0.05271	0.14659
% ala coverage	0.18744	-0.75048	-0.42986
Sclerified portion of leaf tip	0.04654	0.53506	-0.72654
Leaf number	0.54509	0.51899	0.12401
Velum coverage	-0.81467	0.36054	-0.04755

*socia*, and *I. steyermarkii* (nom. nud.). Examination of the holotypes of *I. lechleri*, *I. glacialis* and *I. socia* (all from the Andes of southern Peru or Bolivia) was performed initially to determine whether or not these taxa should be included in the subsequent analyses. Both *I. glacialis* and *I. socia* were determined to be synonyms of *I. lechleri*, which in turn was determined to be distinct from the northern Andean taxa. For a complete discussion of the variability and synonymy of the *I. lechleri* complex see Hickey (1994), but a brief discussion of our rationale for excluding these taxa follows. The synonymy of *Isoetes socia* and *I. lechleri* has been proposed by all authors except Fuchs-Eckert (1982, 1992) since the former's publication. The type specimen of *I. socia* (Lechler 1937b, B!) consists of a single immature plantlet and a few isolated sporophylls. These materials were apparently removed from Lechler 1937 (the type of *I. lechleri*) and undoubtedly represent a vegetative offset from *I. lechleri*, a species in which vegetative reproduction is common (Hickey, 1994). The type of *I. glacialis* (Asplund 4041, isotype B!) is but one of the many morphotypes of the highly variable *I. lechleri* and is clearly distinct, both in morphology and distribution, from Cuatrecasas 19117, the basis for *I. arumiana*.

Sixty-three collections were used in the PCA, including all of the type specimens except those of *Isoetes glacialis*, *I. lechleri*, and *I. socia*. Eigenvectors for the first three principal components are given in Table 3; the first two principal components accounted for 36% and 23%, respectively, of the total variation. A plot of principal components one vs. two (Fig. 1) reveals six potentially distinct groups, which will be referred to in subsequent discussion by number. Clusters 1, 2 and 3 contain single collections: the type of *I. cleefii*, the "type" of *I. arumiana* (nom. nud.) and the type of *I. rimbachiana* respectively. Cluster 4 contains the type collections of *I. karstenii*, *I. palmeri*, *I. colombiana*, and *I. steyermarkii* (nom. nud.), paratypes of *I. palmeri* and *I. cleefii*, and many additional collections. Cluster 5 consists of a group of eight collections without an associated type specimen. Cluster 6 contains a group of five collections without an associated type specimen. The constituents of each of these groups were examined to determine which characters separated each group from the others and to determine whether or not these groups constitute morphologically definable taxa.



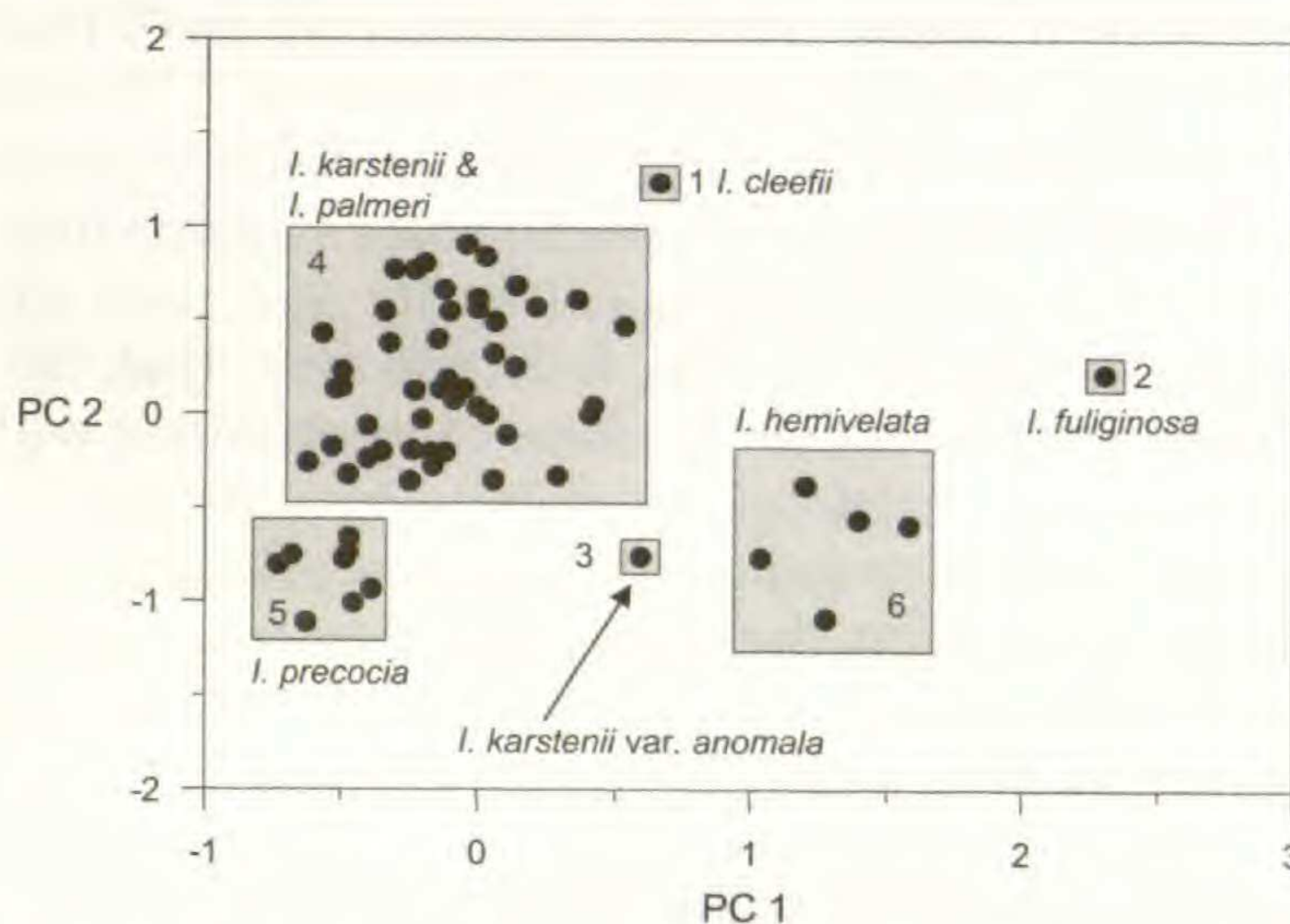


FIG. 1. Scatter plot of principal component one vs. principal component two based on morphological data. Clusters of associated specimens are indicated and numbered. 1 = *I. cleefii*; 2 = *I. fuliginosa*; 3 = *I. karstenii* var. *anomala*; 4 = *I. karstenii* + *I. palmeri*; 5 = *I. precocia*; 6 = *I. hemivelata*.

Cluster 1 (the holotype of *I. cleefii*) appears quite distinct from the remaining clusters in Fig. 1, yet examination of the characters suggests that the separation between clusters 1 and 4 is tenuous. Cluster 1 is separated from cluster 4 along principal component (PC) 2 by leaf length, leaf width and leaf number, yet character by character, the type of *I. cleefii* fell within the range of values of the individuals in cluster 4. The type collection of *I. cleefii* has longer, wider, and more numerous leaves than the average member of cluster 4. However, some collections in cluster 4 have values greater than or equal to those of the type of *I. cleefii* for some of these characters, but not for all. It appears that the combination of high values for all of these characters serve to separate the type of *I. cleefii* from the collections in cluster 4.

Cluster 2 contains the single collection, *Cuatrecasas 19117*, which is the basis of *I. arumiana* (nom. nud.). This collection is separated from the remaining collections along PC 1 by differences in velum coverage, megaspore diameter, and microspore length. In addition, the plant is larger than any other collection in this group, and the ligule is also unusually large (ca. 7 mm high) relative to the other species in this complex. This combination of character states makes this collection the most distinctive of those analyzed, and therefore we suggest that it deserves species status. To avoid further nomenclatural and taxonomic confusion caused by the invalid publication and subsequent synonymy of *I. arumiana* (nom. nud.), we propose this taxon be recognized as *Isoëtes fuliginosa* sp. nov.

Cluster 3 contains the single collection, *Rimbach 171*, the type of *Isoëtes rimbachiana* (= *I. lechleri* var. *anomala*). Differentiation of this collection from *I. karstenii* (from which it is otherwise nearly indistinguishable) by Palmer (1932) was based primarily on velum coverage, which also separates it from the collections of cluster 4 along PC 1 in our PCA. While velum coverage is



often considered to be a highly plastic character, we have found velum coverage to be uniformly complete in all collections of *I. karstenii*. Thus, the presence of an incomplete velum, along with the disjunct geographical placement of this collection at Mt. Chimborazo, Ecuador suggests that this collection represents a potentially unique biological entity and thus should be maintained as a distinct taxon. We do not feel, however, that the distinction is sufficient to warrant specific status. Because of its affinities with *I. karstenii* it should be recognized as *I. karstenii* var. *anomala* comb. nov.

Cluster 4 is a large and heterogeneous collection. Along with a large number of other collections, it contains the holotypes of *Isoëtes karstenii*, *I. colombiana*, *I. steyermarkii* (nom. nud.), and *I. palmeri*, as well as paratypes of both *I. palmeri* and *I. cleefii*. Due to this heterogeneity and the dubious separation of *I. cleefii*, we performed additional analyses on this group (see below).

Cluster 5 contains a group of eight collections without an associated type specimen. While placed close to cluster 4 (Fig. 1), this group of collections exhibits a unique set of character states that set it apart from the remaining specimens. Specifically, the PCA separates them from other specimens based on their diminutive size (leaf length, leaf width, and leaf number) at maturity, and the reduction of the corneous leaf tip. These specimens are also ecologically differentiated as they are found solely in ephemeral pools, not permanent lakes and ponds as are the other species of this group. We propose that this taxon be recognized as *Isoëtes precocia* sp. nov.

Cluster 6 contains a group of five collections without an associated type specimen. These collections are distinguished from the remaining collections along PC 1 by velum coverage, megaspore diameter, and microspore length. This group of specimens is also unique within the complex in having trigonal rather than terete leaves. Although similar to cluster 2 (*Isoëtes fuliginosa*) in velum coverage and spore dimensions, the two are easily distinguishable based on leaf cross-sectional shape, leaf length, ala length / leaf length ratio, leaf number, and the length of the sclerified portion of the leaf tip. We propose that this unique taxon be recognized as *Isoëtes hemivelata* sp. nov.

To examine the possibility that additional undetected groups existed within cluster 4 and to determine the status of *Isoëtes cleefii*, two-dimensional scatter plots of all combinations of characters were produced for those collections in clusters 1 and 4. The scatter plot of leaf length vs. leaf width at the mid-point (Fig. 2) reveals two distinct groups with different character trends: one displaying relatively long, slender leaves, the other with relatively short, thick leaves. The group with relatively long, slender leaves contains type and paratype collections of *I. palmeri* and *I. cleefii* (Fig. 2) as well as a number of additional collections. The other group (Fig. 2), with relatively short, thick leaves, contains the type collections of *I. karstenii*, *I. colombiana*, and *I. steyermarkii* (nom. nud.) as well as a number of additional collections. Linear regression analyses of these specimens, individually and taken together support our inferences that these two groups are distinct. If all specimens in Fig. 2 are included in a regression analysis, the  $r^2 = 0.0009$  ( $P = 0.85$ ) indicates a lack of significant correlation. If however, the two groups (above and below



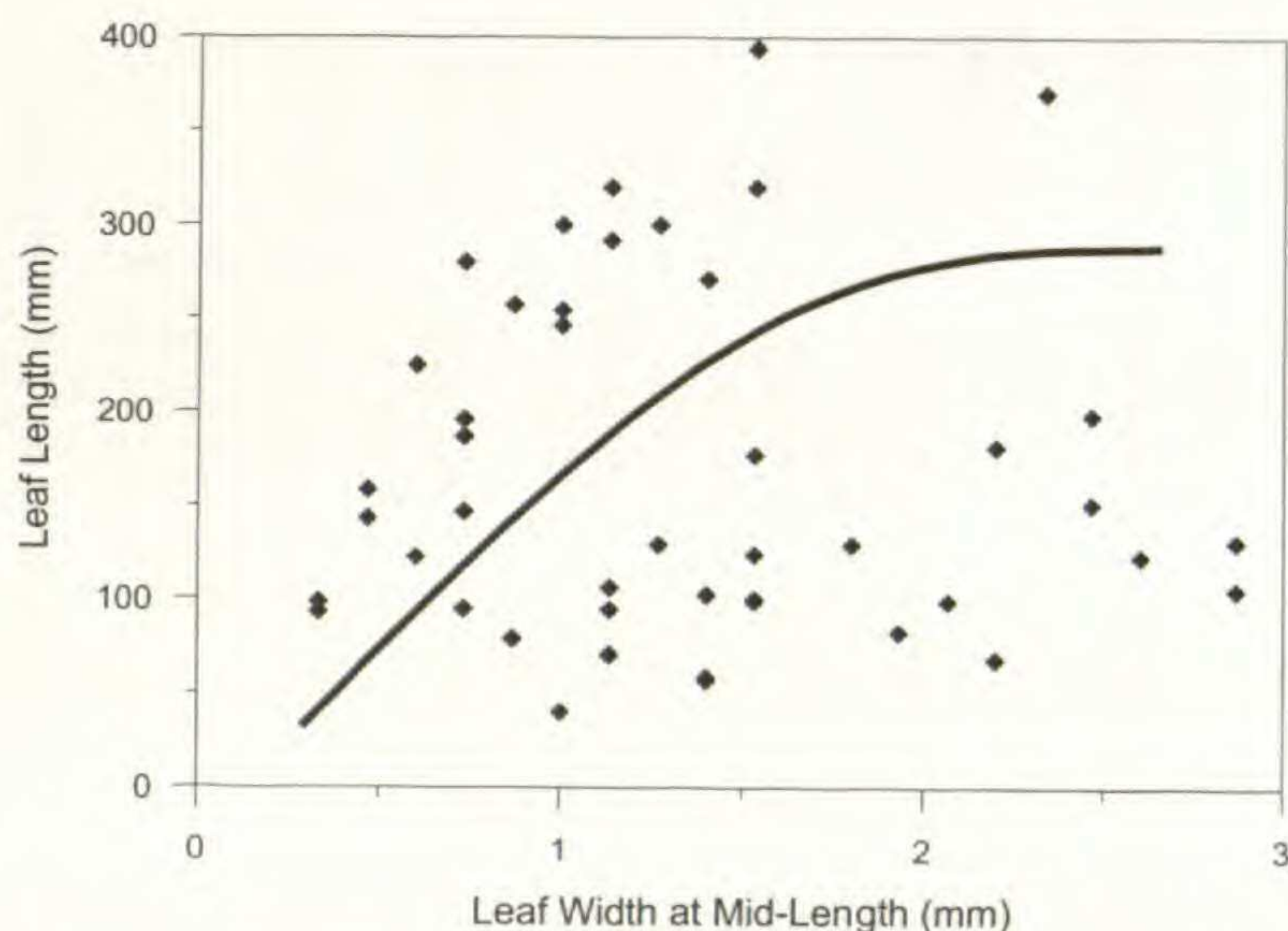


FIG. 2. Two-dimensional scatter plot of leaf length (mm) vs. leaf width (mm) for those specimens included in cluster 4 of the PCA (Fig. 1). The line divides the relatively long, narrow-leaved specimens (*I. palmeri*) from the relatively short, stout-leaved specimens (*I. karstenii*).

the line in Fig. 2) are analyzed separately, values of  $r^2 = 0.70$  ( $P = 1.2 \times 10^{-6}$ ) and  $r^2 = 0.25$  ( $P = 0.012$ ), respectively, are obtained. These values indicate that leaf length and width are significantly correlated within groups, thus lending support to our hypothesis that they are biologically distinct. The separation of these two very similar groups appears to have been obscured in the initial PCA by the inclusion of such a morphologically diverse selection of specimens. The range of variation encompassed by the initial PCA was much larger than the variation between groups within cluster 4, therefore the entire range of variation encompassed by cluster 4 was confined to a small portion of the entire PCA space. A more fine-scaled analysis was then required to detect these differences. In addition to the separation of the two groups described above, this analysis clarifies the position of the type collection of *I. cleefii* as an extreme individual along a continuum (Fig. 2).

On the basis of these analyses we delimit two additional species. The group with relatively long, slender leaves includes the type collections of *Isoëtes palmeri* and *I. cleefii*. These two taxa were published simultaneously (Fuchs-Eckert, 1981a,b), and thus neither has priority. We propose that this taxon be recognized as *I. palmeri* because the type specimen is more representative of the species overall. The group with relatively short, wide leaves includes the type collections of *I. karstenii*, *I. colombiana*, and *I. steyermarkii* (nom. nud.). The name *I. karstenii* was the first of these to be published (Braun, 1862) and therefore has priority.

In order to document the variation within these species, we calculated the mean and range of each character (Table 4). Although leaf length / leaf width at the mid-point ratio was not included as a character in the PCA (because both leaf length and leaf width were included as separate characters) it is an important character in discriminating *Isoëtes karstenii* from *I. palmeri* and was therefore included in descriptive statistics. Additionally, as chromosome num-



TABLE 4. Distinguishing character states for members of the *Isoetes karstenii* complex. For each taxon N = the number of collections sampled. For each taxon and character the range and mean  $\pm$  standard error (in parentheses) are presented.

	<i>I. karstenii</i> (N = 24)	<i>I. palmeri</i> (N = 22)	<i>I. precocia</i> (N = 8)	<i>I. hemivelata</i> (N = 8)	<i>I. fuliginosa</i> (N = 1)
Leaf Number	8-(13 $\pm$ 3.5)-21	7-(14 $\pm$ 4.6)-22	6-(7 $\pm$ 1.6)-11	11-(13 $\pm$ 1.8)-16	25
Leaf length (mm)	40-(115 $\pm$ 53.8)-251	93-(230 $\pm$ 90.2)-394	37-(52 $\pm$ 19.0)-94	55-(129 $\pm$ 56.7)-184	255
Leaf width at mid-length (mm)	0.9-(1.6 $\pm$ 0.4)-2.4	0.5-(1.0 $\pm$ 0.4)-1.4	0.5-(0.7 $\pm$ 0.2)-0.9	1.2-(1.9 $\pm$ 0.5)-2.4	1.9
Leaf length/leaf width ratio	36-(75 $\pm$ 31.4)-157	119-(241 $\pm$ 54.1)-350	46-(80 $\pm$ 34.5)-157	46-(64 $\pm$ 16.2)-82	134
Ala length/leaf length ratio	0.14-(0.26 $\pm$ 0.06)-0.38	0.11-(0.19 $\pm$ 0.04)-0.28	0.26-(0.30 $\pm$ 0.04)-0.36	0.28-(0.34 $\pm$ 0.05)-0.41	0.15
Corneous portion of leaf apex (mm)	0.7-(1.8 $\pm$ 0.06)-3.2	0.8-(1.4 $\pm$ 0.5)-2.4	0.1-(0.2 $\pm$ 0.1)-0.5	0.8-(1.2 $\pm$ 0.3)-1.6	0
Velum coverage (%)	>90%	>90%	>90%	35-75%	50-75%
Megaspore diameter ( $\mu$ m)	395-(479 $\pm$ 110.9)-507	388-(488 $\pm$ 119.9)-551	388-(442 $\pm$ 37.2)-495	513-(565 $\pm$ 49.1)-622	600-700
Microspore length ( $\mu$ m)	25-(30 $\pm$ 3.7)-38	26-(31 $\pm$ 3.1)-36	27-(29 $\pm$ 1.1)-30	37-(39 $\pm$ 1.3)-40	48
Chromosome number	2n = 22	2n = 44	2n = 22	2n $\geq$ 44	2n $\geq$ 44



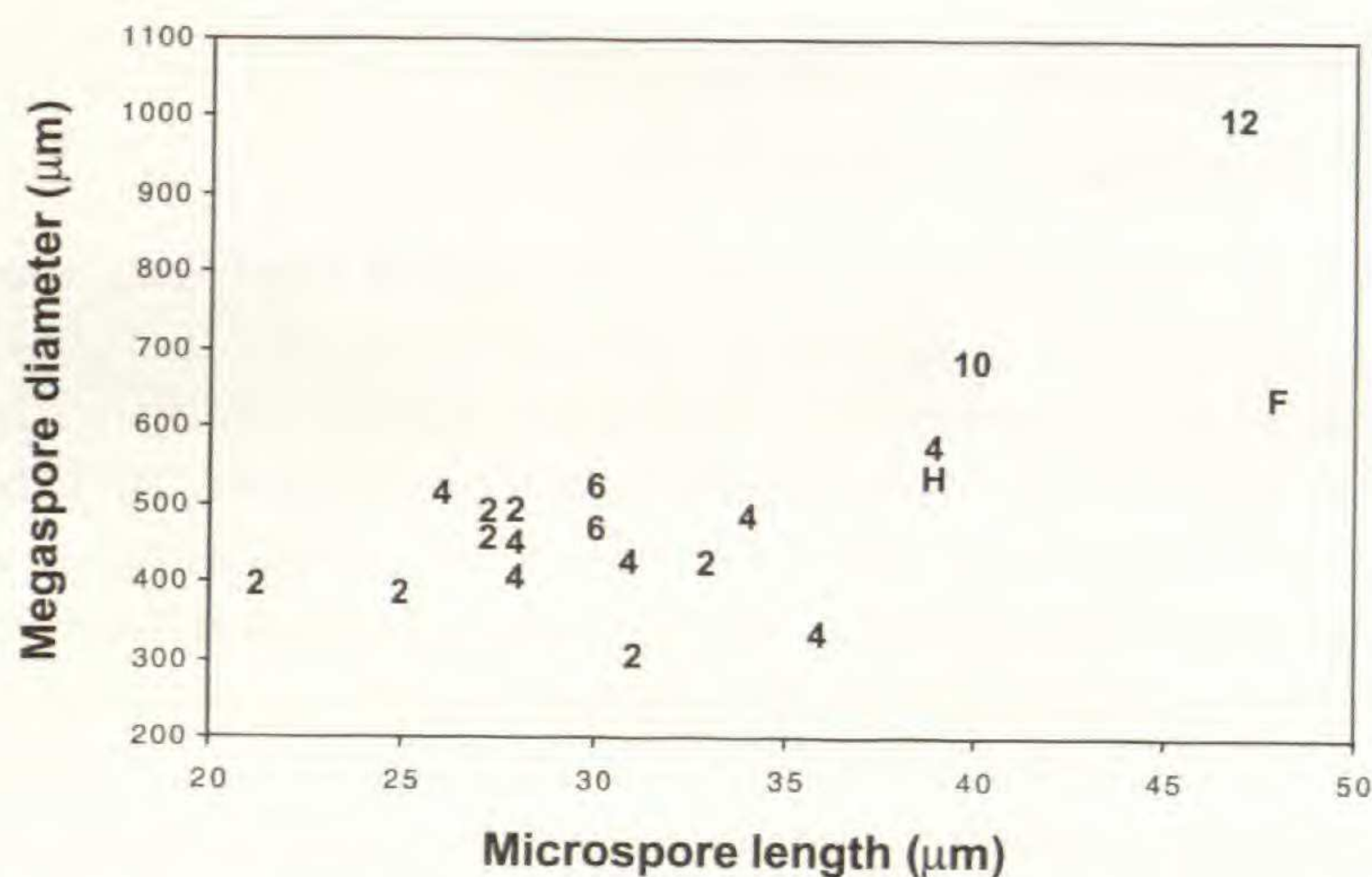


FIG. 3. Two-dimensional scatter plot of microspore length ( $\mu\text{m}$ ) vs. megaspore diameter ( $\mu\text{m}$ ) for species of *Isoetes* with known chromosome numbers. Each point represents a single species with its chromosome number identified by number (e.g., "2" =  $2x = 22$ , "4" =  $4x = 44$ , etc.). The two unknown species are identified by letter ("H" = *I. hemivelata*; "F" = *I. fuliginosa*).

bers are important in distinguishing taxa (see below), these data are also provided in Table 4.

**CYTOLOGY.**—Chromosome counts were performed for *Isoetes karstenii*, *I. palmeri*, and *I. precocia* (Table 2). It was found that *I. karstenii* and *I. precocia* were diploids ( $2n = 22$ ) and *I. palmeri* was tetraploid ( $2n = 44$ ). In addition to numerous counts of  $2n = 44$  for *I. palmeri*, a single individual from one population of *I. palmeri* was found to be a triploid ( $2n = 33$ ). This individual presumably represents a hybrid between *I. palmeri* and a diploid species, such as *I. karstenii* or *I. precocia*, both of which are sympatric with *I. palmeri* in the area of this population. These cytological data support the findings of the morphological analyses in further distinguishing *I. karstenii* and *I. precocia* from *I. palmeri*.

Live material was not available for *Isoetes fuliginosa* or *I. hemivelata*. The relatively large size of the mega- and microspores of these taxa, however, suggest that they are most likely polyploid. We attempted to estimate the chromosome numbers of these taxa by comparing their spore sizes with taxa of known chromosome number. A scatter plot of megaspore diameter vs. microspore length was produced with individuals identified by chromosome number (Fig. 3). Individual ploidy clusters show overlap with adjacent ploidy clusters and thus it is not possible to confidently characterize the chromosome numbers of the unknown species. Despite this, these data clearly indicate that these taxa are polyploid, both being well out of the range of known diploids. *Isoetes fuliginosa* appears to be a high-level polyploid, probably at least a hexaploid. *Isoetes hemivelata* is more difficult to characterize because it falls out near taxa of three different ploidy levels, yet the data suggest that it is at least a tetraploid, possibly higher.



## DISCUSSION

## Taxonomy and Analyses

As a result of our morphological and cytological analyses, we recognize the following taxa within the *I. karstenii* species complex: *I. fuliginosa*; *I. hemivelata*; *I. karstenii* var. *karstenii*; *I. karstenii* var. *anomala*; *I. palmeri*; and *I. precocia*. The details of this taxonomic history are given in Table 1.

The conclusions presented here are based on evaluation of morphological characters using multivariate analyses to derive distinct groups of populations. In the case of these taxa, traditional approaches using intuitive taxonomic assessments have led to undue lumping (placing all taxa in *I. lechleri*) or splitting (recognition of up to eight taxa within this complex by Fuchs-Eckert 1982). Many workers have discussed the morphological plasticity in *Isoëtes*, yet few have emphasized the fact that circumscribing that variation is imperative for understanding species delimitations (but see Kott and Britton, 1985). Evidence presented in this study supports the utility of quantitative vegetative characters in delimiting species, especially when those characters are examined using a multivariate approach.

## Cytology and Speciation

Speciation in *Isoëtes* appears to occur primarily via two pathways: (1) allopatric speciation of populations of the same ploidy level, and (2) speciation via allopolyploidy (Taylor and Hickey, 1992). Cytological and morphological data suggest that both modes of speciation have occurred in the *I. karstenii* complex.

Reticulate evolution involving diploids and allopolyploids has been well documented in *Isoëtes* (Taylor et al., 1985; Hickey et al., 1989; Taylor and Hickey, 1992), yet autopolyploidy is apparently rare, having been reported only once (Rumsey et al., 1993). Allopolyploids in *Isoëtes* are generally characterized by an intermediate morphology with respect to the parental species, especially in megaspore morphology, and additive chromosome numbers and allozyme banding patterns (Taylor and Hickey, 1992). Autopolyploids would be expected to strongly resemble the parental species in all characters except chromosome number and spore size.

Because the tetraploid *Isoëtes palmeri* has completely laevigate megaspores and *Isoëtes* allopolyploids appear to always have intermediate megaspore surface morphology only those diploid taxa with laevigate megaspores should be considered as possible parents. Based on the results of this study only *I. karstenii* and/or *I. precocia* are likely to be involved.

Although conclusive evidence to address the auto- vs. allopolyploid origin of *I. palmeri* is unavailable, the data do suggest that the allopolyploid hypothesis is more plausible for several reasons. First, as mentioned, autopolyploidy is rare in *Isoëtes*, but allopolyploidy is frequent. Second, although *I. karstenii*, *I. precocia*, and *I. palmeri* are all morphologically similar, *I. palmeri* is intermediate for some characters (e.g., leaf width, length of corneous portion of the



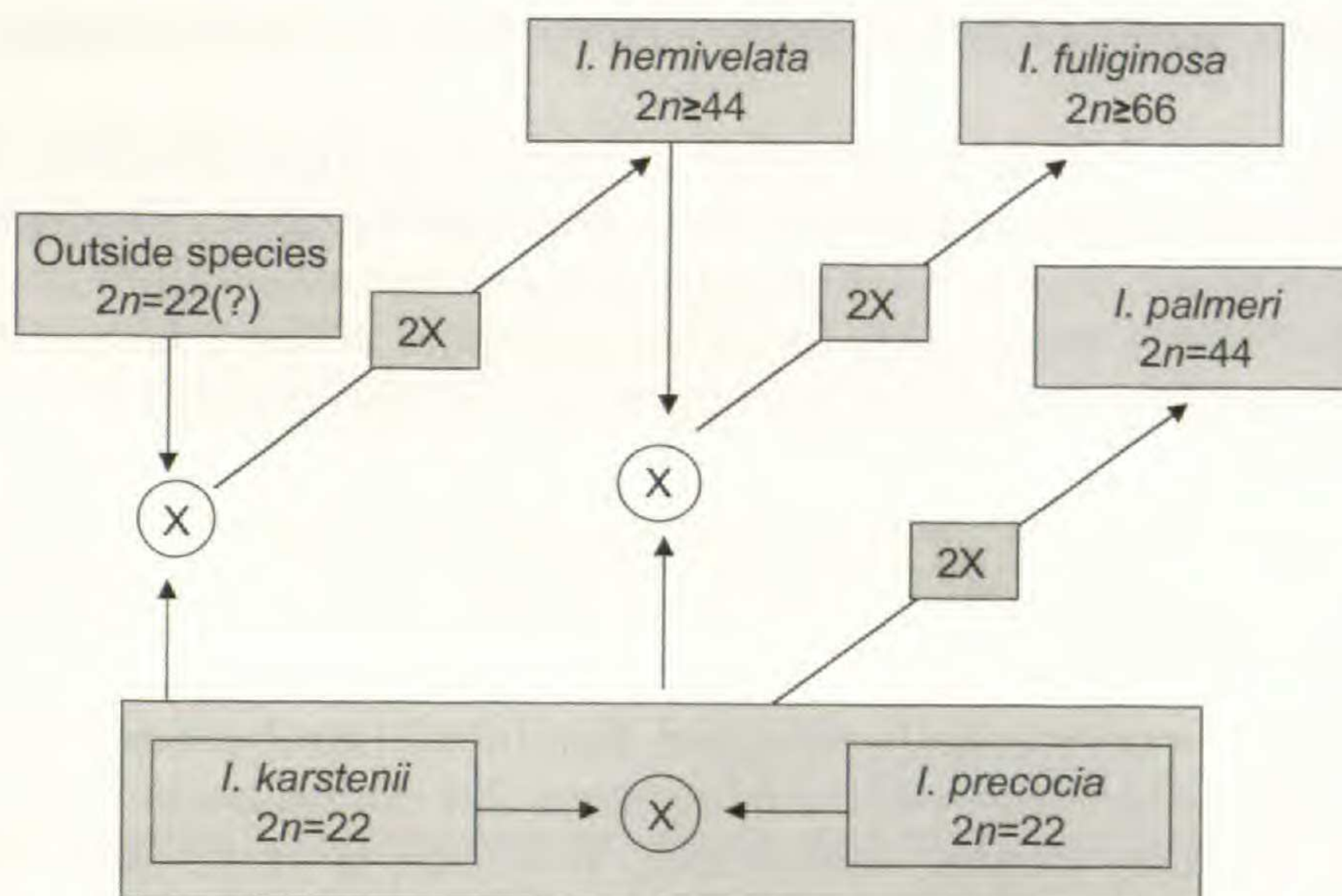


FIG. 4. Hypothesized relationships among the species of the *I. karstenii* complex. Circled  $\times$ 's represent a hybridization event; boxed  $2\times$ 's represents a chromosome doubling event.

leaf). Finally, the presence of a triploid in one of the populations of *I. palmeri* suggests that hybridization occurs between *I. palmeri* and one (or both) of the diploids. Although this does not bear directly on the origin of *I. palmeri*, it suggests that hybridization does occur in this group.

*Isoëtes karstenii* and *I. precocia* are both diploids and therefore have their origins in divergent (rather than reticulate) speciation. These two species exist in different habitats: *I. karstenii* is evergreen and is found in permanent lakes and ponds while *I. precocia* is ephemeral and is confined to small, seasonal pools which fill during the wet season and desiccate during the dry season. Given the phenetic resemblance and geographic sympatry of these two taxa they are probably closely related. Whether one gave rise to the other or they are each descendants of a now extinct taxon is beyond the scope of this study.

### Species Relationships

Although further taxonomic and biosystematic work needs to be done, we offer a preliminary hypothesis regarding the relationships among the species in this complex (Fig. 4). The base of these relationships rest on the two diploid species, *I. karstenii* and *I. precocia*. Hybridization between these two species followed by chromosome doubling in the  $F_1$  results in the allotetraploid, *I. palmeri*.

The origin of *I. hemivelata* is less clear, but one possibility is hybridization between a member of the *I. karstenii* complex and a species outside the complex, possibly *I. andina* or one of several undescribed Colombian or Ecuadorian species (Hickey, unpublished data). The evidence to support this includes: *I. hemivelata*'s possession of character states derived from the *I. karstenii* com-



plex and from the *I. andina* complex, along with its undoubtedly polyploid nature.

The origin of *I. fuliginosa* possibly occurred by hybridization between *I. hemivelata* and another member of the *I. karstenii* complex. *Isoëtes fuliginosa* is clearly polyploid, shares the character state of reduced velum coverage with *I. hemivelata*, and is larger than the other members of the *I. karstenii* complex, but in other respects maintains the characters of the complex.

TAXONOMIC TREATMENT

*Isoëtes karstenii* Complex

Corm globose to horizontally elongate, two lobed (rarely three), size variable; dichotomously branching roots arising from the circumbasal fossa(e). Leaves variable in number, length, and width, flexuous or stiff; alae chartaceous, brown to black, extending upward to 50% of total leaf length, ala apices attenuate, acute, truncate or free; subula terete to trigonal, olive green to brown; leaf apices corneous, typically spatulate. Scale leaves, phyllopodia, stomates and peripheral fibrous bundles not seen. Sporangia (ob)ovate to elliptic, tan, concolorous to spotted, basal. Velum incomplete to complete. Ligule deltate to trullate. Labium absent or reduced to a small transverse ridge on the lower lip of the foveola. Megaspores white, 350–700 µm in diameter, laevigate on both proximal and distal faces. Microspores cocoa brown, 25–55 µm in length, echinate. Vegetative reproduction not observed.

As discussed previously, these taxa have often been placed in synonymy with, or regarded as varieties of, *I. lechleri*. They can be distinguished from that species, however, on the basis of ala length/leaf length ratio (up to 50% in the *I. karstenii* complex vs. 50–75% in *I. lechleri*), ala apex morphology (attenuate to free in the *I. karstenii* complex vs. always attenuate in *I. lechleri*), vegetative reproduction (common in *I. lechleri* vs. absent in the *I. karstenii* complex), and distribution (*I. karstenii* complex in Ecuador, Colombia and Venezuela vs. *I. lechleri* in southern Peru and Bolivia). Additionally, the only member of the *I. karstenii* complex which strongly resembles *I. lechleri* is *I. karstenii*. These two taxa can be further differentiated on the basis of chromosome number: *I. karstenii* has  $2n = 22$  and *I. lechleri* has  $2n = 44$  (Hickey, 1994).

KEY TO SPECIES OF THE *I. KARSTENII* COMPLEX

- 1. Velum covering 25–75% of the sporangium
  - 2. Alae extending 10–20% up the leaf length; leaves 150–310 mm long; velum covering 50–75% of the sporangium; microspores 45–55 µm long; megaspores 600–700 µm in diameter . . . . . 1. *I. fuliginosa*
  - 2. Alae extending 25–45% up the leaf length; leaves 40–200 mm long; velum covering 30–50% of the sporangium; microspores 25–45 µm long; megaspores 300–650 µm in diameter
  - 3. Megaspores 300–450 µm in diameter; microspores 30–40 µm long; subula terete . . . . . 4b *I. karstenii* var. *anomala*



- 3. Megaspores 500–650  $\mu\text{m}$  in diameter; microspores 35–45  $\mu\text{m}$  long; subula trigonal  
 ..... 2. *I. hemivelata*
- 1. Velum covering >90% of the sporangium
  - 4. Leaf apices not or only slightly sclerified (the sclerified part < 0.5 mm long); leaves less than 100 mm long, less than 1.0 mm wide at the middle; plants seasonally ephemeral ..... 3. *I. precocia*
  - 4. Leaf apices distinctly sclerified (the sclerified part 0.7–3.2 mm long); leaves 40–400 mm long, 0.5–2.4 mm wide at the middle; plants seasonally persistent
    - 5. Leaves 25–200 mm long and 1.0–2.5 mm wide with a leaf length/leaf width ratio of 30–160;  $2n = 22$  ..... 4a. *I. karstenii* var. *karstenii*
    - 5. Leaves 100–400 mm long and 0.5–1.5 mm wide with a leaf length/leaf width ratio of 110–350;  $2n = 44$  ..... 5. *I. palmeri*

1. *Isoëtes fuliginosa* R. L. Small and Hickey, **sp. nov.** (Fig. 5–7; 14–15).

Type: “Colombia. Departamento del Cauca: Cordillera Central, Lagunilla de las Casitas, 3700 m alt., 3 Dec 1944” *Cuatrecasas 19117* (Holotype: F!; Isotypes: COL!, F!, G-2!, GH!, MO!, MU!, US!).

*Isoëtes arumiana* Fuchs-Eckert, nom. nud. Proc. Kon. Ned. Akad. Wetensch. C. 85(2):259. 1982. Based on *Cuatrecasas 19117* (US!)

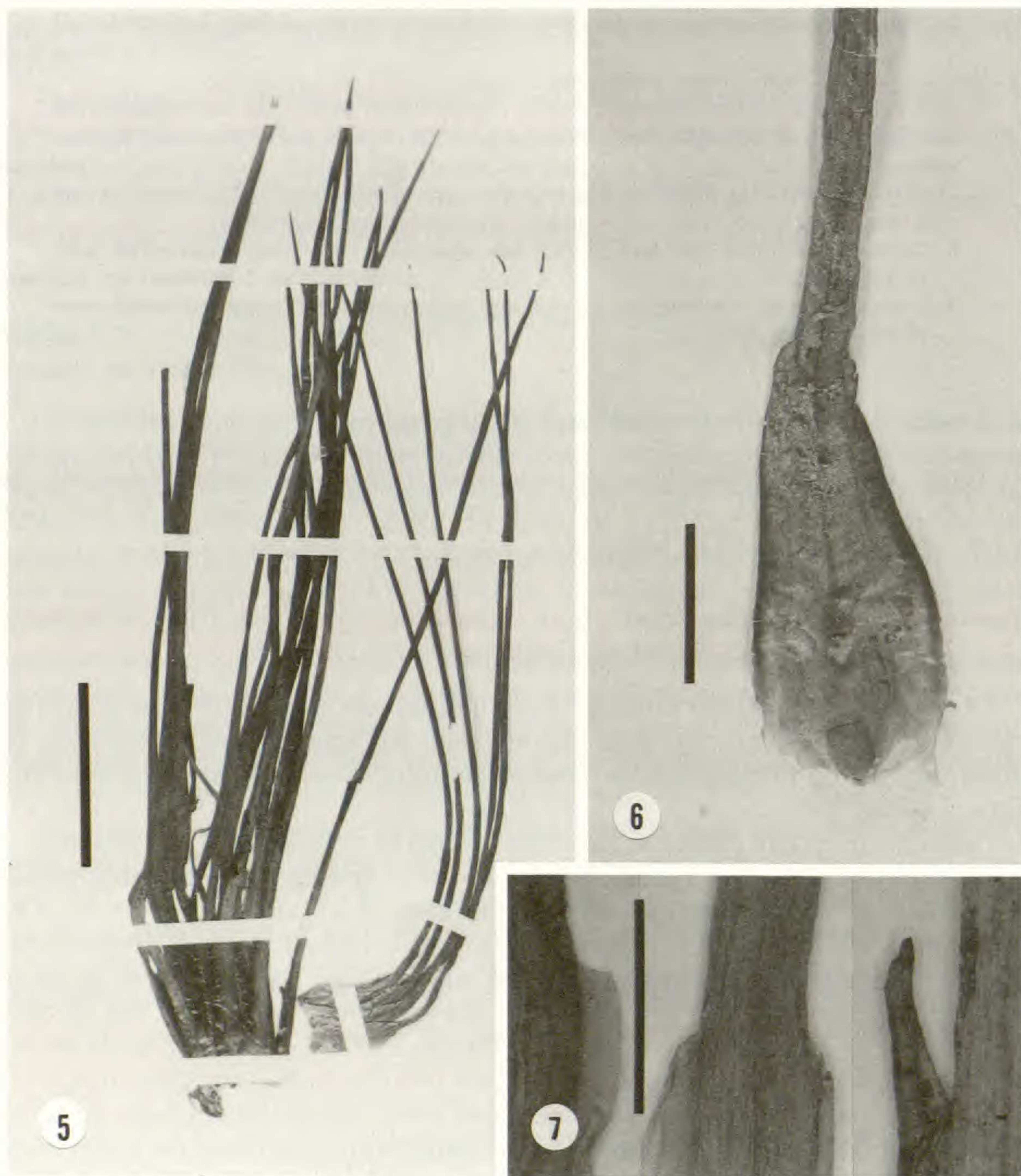
Ob statura magna, velo incompleto et megasporis grandibus inter omnes *Isoëtes* species ab Andibus septentrionalibus peculiaris; *Isoëtes karstenii* A. Braun tangit, ob megasporis laevibus et ob apicibus aliis truncatis versus liberis.

Corm horizontally elongate, two-lobed, 9–10 ( $x = 9.7$ ) mm high, 24–25 ( $x = 24.7$ ) mm wide; dichotomously branching roots arising from the circumbasal fossa. Leaves 18–34, flexuous, to 310 mm long, 8–12 mm wide at base, 1.5–2.5 mm wide at mid-length, leaf length/width ratio 134; alae chartaceous, brown to blackened, extending 11–17% of total leaf length, apices acute to truncate; subula terete in cross-section, olive green, apex obtuse, not or only slightly corneous at the tip; peripheral fibrous bundles, stomates, scale leaves and phyllopodia absent. Sporangia obovate to ovate, basal, unpigmented, 5.5–7.8 ( $x = 7.2$ ) mm long, 4.5–5.8 ( $x = 5.2$ ) mm wide. Velum incomplete, covering 50–75% of the sporangial surface. Ligule deltate and auriculate, ca. 7 mm high, 5 mm wide. Labium absent or reduced to an extremely small transverse ridge on lower lip of the foveola. Megaspores immature, but ca. 600–700  $\mu\text{m}$  in diameter, laevigate on both proximal and distal faces. Microspores cocoa brown, 45–55 ( $x = 48.2$ )  $\mu\text{m}$  in length, 30–40 ( $x = 35.4$ )  $\mu\text{m}$  wide, echinate. Chromosome number unknown.

*Isoëtes fuliginosa* is known only from the type collection which was taken from a small lake or pond (“lagunilla”) at 3700 m altitude in the Cordillera Central, Departamento Cauca, Colombia (Fig. 8). This collection was made in December and contains both microspores and megaspores; however, the megaspores are immature. Further collections are necessary to characterize the phenology and full range of morphological variation of this species.

Despite the immaturity of this collection, *Isoëtes fuliginosa* is distinct from





FIGS. 5–7. *Isoetes fuliginosa* (Cuatrecasas 19117). FIG. 5. Habit view showing reduced alae (F #1351470). Bar = 5 cm. FIG. 6. Microphyll base showing reduced alae and partial velum (US). Bar = 1 cm. FIG. 7. Variation in ala apices: truncate, acute and free respectively (F # 1351471). Bar = 5 mm.

other species of *Isoetes* and can be recognized by its incomplete velum, large growth habit and large mega- and microspores. It is clearly related to the other members of the *I. karstenii* complex given its laevigate megaspores and truncate to free ala apices, although the incomplete velum and apparently high ploidy level indicate that an outside influence is also present. The results of the spore analyses show that *I. fuliginosa* is polyploid (probably hexaploid or



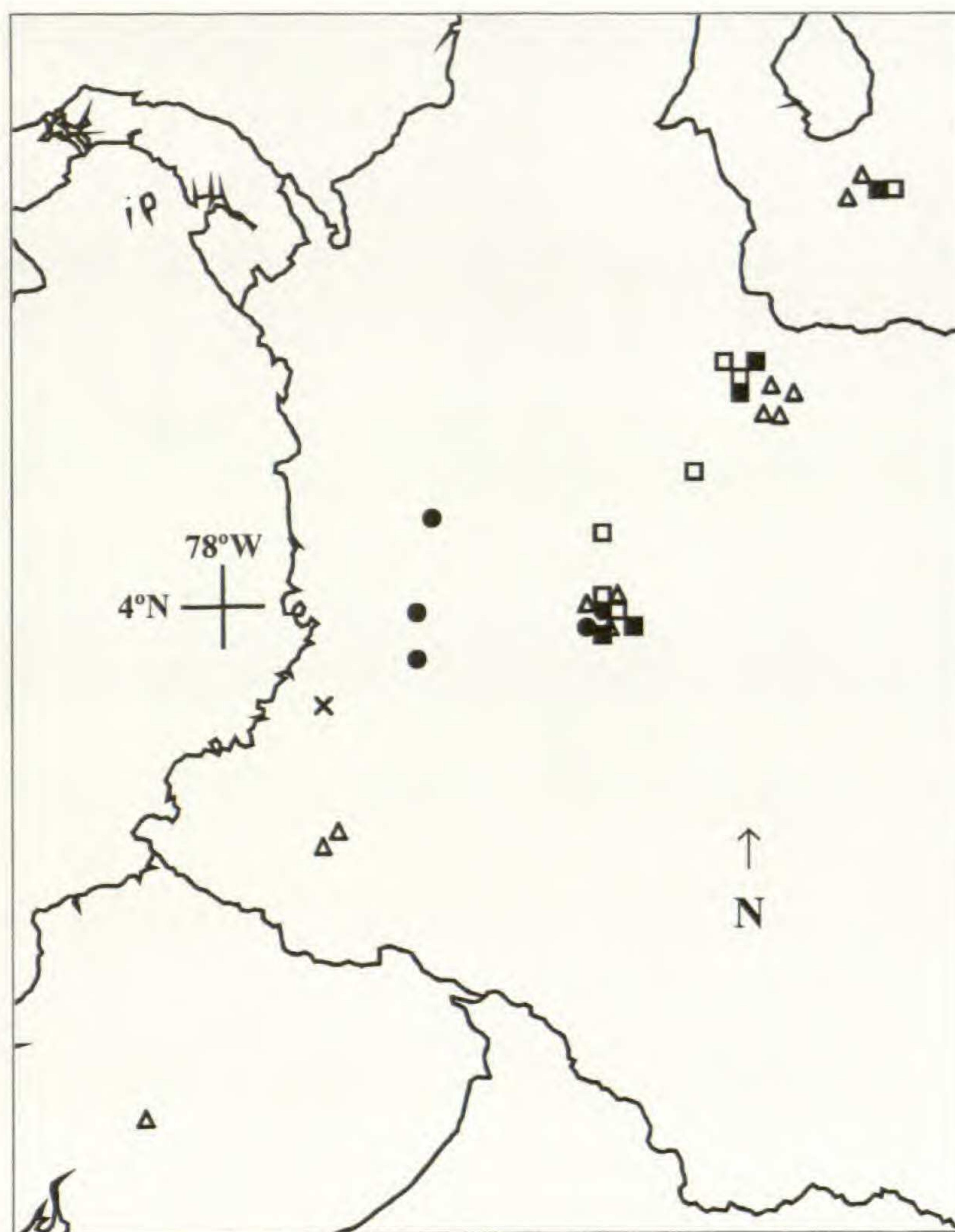


FIG. 8. Distributions of the species of the *I. karstenii* complex. Open triangles = *I. karstenii*, open squares = *I. precocia*, filled squares = *I. palmeri*, filled circles = *I. hemivelata*, and the X = *I. fuliginosa*.

higher) and therefore is likely an allopolyploid derivative of other nothospecies.

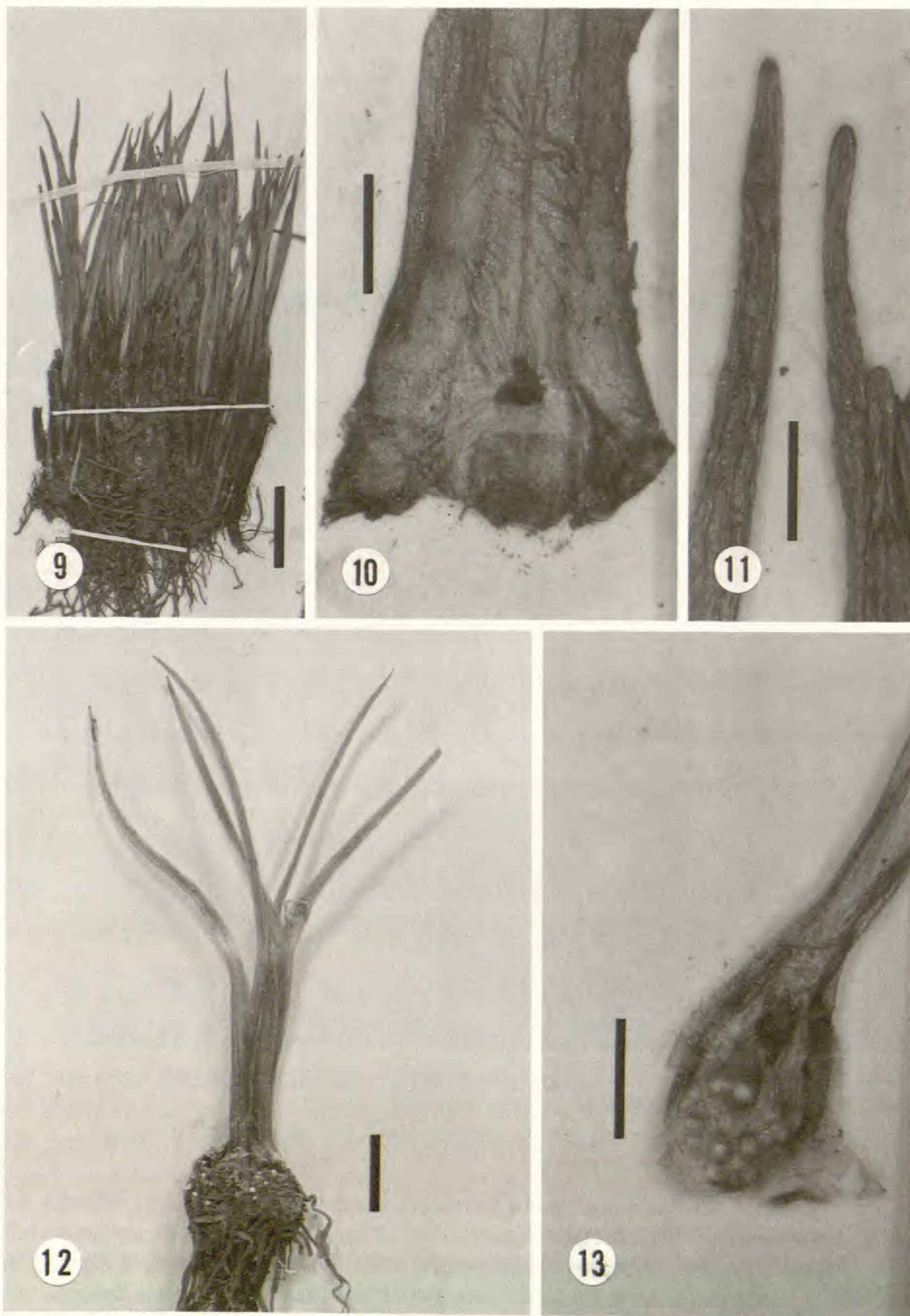
## 2. *Isoëtes hemivelata* R.L. Small and Hickey, **sp. nov.** (Fig. 9–11, 16–17)

Type: "Colombia, Departamento del Valle, Cordillera Central, vertiente occidental; cabeceras de los ríos Tuluá y Bugalagrande: Páramo de Las Vegas, alt 3600–3800 m, 22 March 1946" *Cuatrecasas 20301* (Holotype: F!; Isotypes: M!, NY!, UC!, US!)

Haec species ab Sectione *Isoete karstenii* praeclare distinguitur subulis trigonis et velis 30–50% obtegentibus.

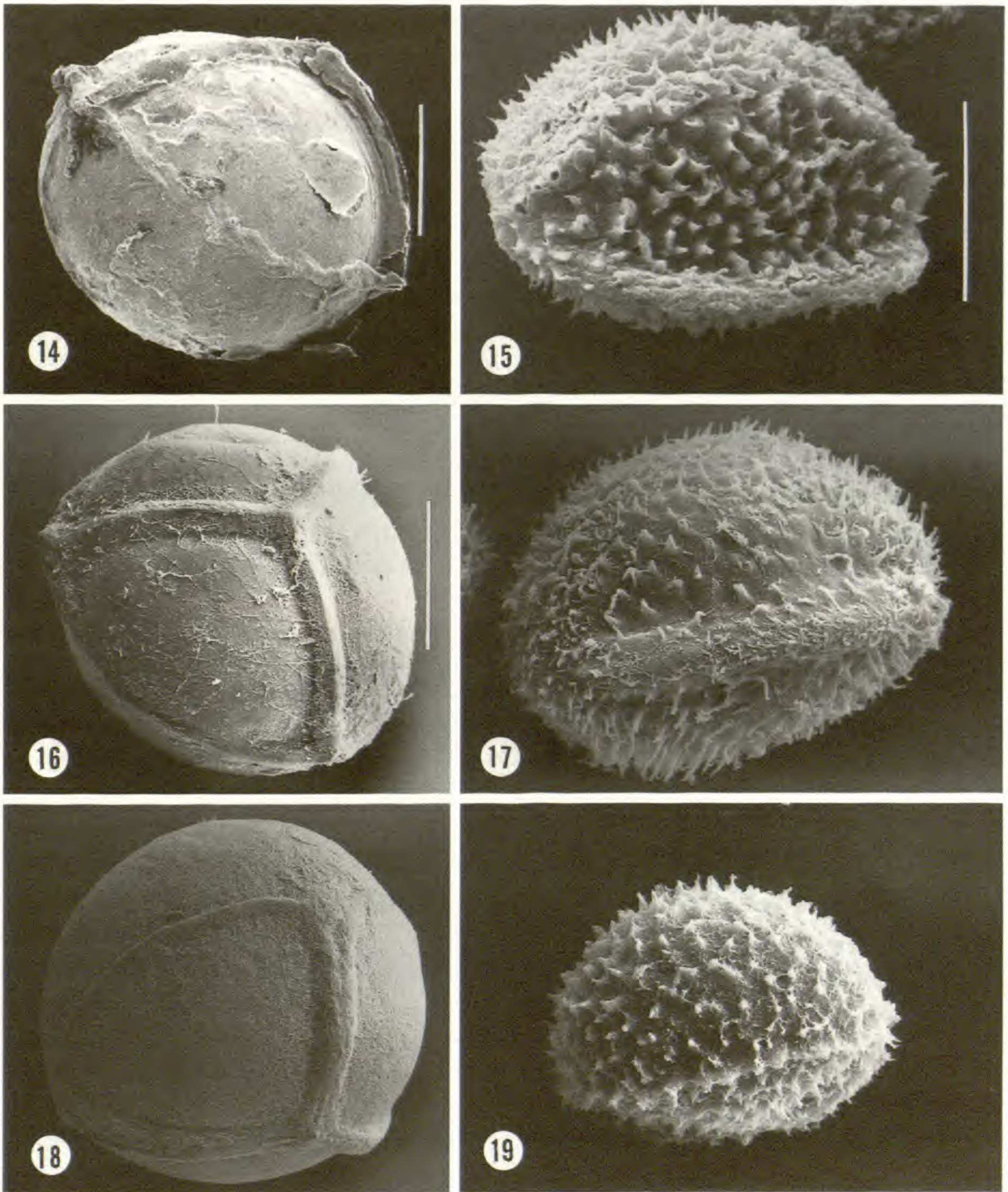
Corm globose, two-lobed, 4–12 ( $x = 6.5$ ) mm high, 6–11 ( $x = 8.1$ ) mm wide; dichotomously branching roots arising from the circumbasal fossa. Leaves 10–25, stiffly erect, to 200 mm long, 4–6 mm wide at base, 1.2–2.4 mm wide at mid-length, leaf length/width ratio 45–82 ( $x = 64$ ); alae chartaceous, dark brown to black, extending 28–51% ( $x = 37\%$ ) of leaf length, apices acute to





FIGS. 9–13. *Isoetes hemivelata* (Cuatrecasas 20301, US) and *I. precocia* (Oberwinkler & Oberwinkler 12968, M). FIG. 9. Habit view of several plants of *I. hemivelata*. Bar = 2 cm. FIG. 10. Leaf base of *I. hemivelata* showing partial velum coverage. Bar = 2 mm. FIG. 11. Subula apices of *I. hemivelata* showing black, corneous apices. Bar = 2 mm. FIG. 12. Habit view of a mature fertile specimen of *I. precocia*. Bar = 1 cm. FIG. 13. Leaf base of *I. precocia* showing a well filled megasporangium with a complete velum and the narrow, parallel alae. Bar = 2 mm.





FIGS. 14–19. *Isoëtes fuliginosa*, *I. hemivelata*, and *I. precocia*. FIGS. 14–15—SEM of spores of *Isoëtes fuliginosa* (Cuatrecasas 19117, US). FIG. 14. Equatorial view of immature megaspore. Bar = 200  $\mu$ m. FIG. 15. Proximal view of microspore showing one of the two proximal surfaces. Bar = 20  $\mu$ m. FIGS. 16–17—SEM of spores of *I. hemivelata* (Cuatrecasas 20301, F). FIG. 16. Proximal view of megaspore. Scale as in Fig. 14. FIG. 17. Proximal view of microspore. Scale as in Fig. 15. FIGS. 18–19—SEM of spores of *I. precocia* (Oberwinkler & Oberwinkler 12968, M). FIG. 18. Near proximal view of megaspore. Scale as in Fig. 14. FIG. 19. Equatorial view of microspore. Scale as in Fig. 15.



free; subula  $\pm$  trigonal, olive green; leaf apices blunt, often spatulate, distal 0.75–1.75 mm of apex corneous; peripheral fibrous bundles, stomates, scale leaves and phyllopodia absent. Sporangia elliptic to obovate, basal, spotted, 1.5–3.2 mm long, 1–2 mm wide. Velum incomplete, covering 30–50 % of the sporangial surface. Ligule shallowly deltate to trullate, to 1.5 mm long, to 2 mm wide. Labium absent or reduced to a transverse ridge on the lower lip of the foveola. Megaspores white, 500–650 ( $x = 560$ )  $\mu\text{m}$  in diameter, laevigate on both proximal and distal faces. Microspores cocoa brown, 35–40 ( $x = 38.5$ )  $\mu\text{m}$  long, 25–30 ( $x = 27.6$ )  $\mu\text{m}$  wide, echinate. Chromosome number unknown.

*Isoëtes hemivelata* grows as a submerged aquatic in permanent lakes and ponds (occasionally streams) between 3300–4300 m. It is distributed throughout the central and southern Departamentos of the Colombian Andes including Meta, Risaralda, Huila, Cauca and Valle (Fig. 8).

Although known from only a few collections, those included in this study were made in January, March, August and October and all contained both mega- and microspores. This suggests that this species is evergreen (as are most Andean *Isoëtes*) and produces both mega- and microspores throughout the year.

*Isoëtes hemivelata* is one of the most distinctive elements within the *I. karstenii* complex given its trigonal subula and incomplete velum. It can be distinguished from *I. karstenii*, *I. palmeri*, and *I. precocia* by these characters and its larger spores. It can be distinguished from *I. fuliginosa* by its trigonal subula, smaller spores, and greater ala length / leaf length ratio.

Analyses of spore size suggest that *I. hemivelata* is polyploid. The characters it shares with the *I. karstenii* complex indicate that one of the parental species comes from this complex; however, the trigonal subula and reduced velum coverage must have come from a source outside this complex. This suggests hybridization and polyploidization. One likely parent is *I. andina* Spruce ex Hook., a sympatric species with strongly trigonal leaves and an incomplete velum. One collection seen by us (*Cuatrecasas 19058* F!, GH!, US!) apparently represents a collection of hybrids between a member of the *I. karstenii* complex and *I. andina*. This collection is highly variable for most characters and spore abortion is evident. While most megaspores of this collection are slightly to distinctly papillate, some are almost laevigate and all individuals of the collection have  $\pm$  trigonal leaves. Chromosome doubling in a hybrid such as this, or backcrossing to the *I. karstenii* complex parent could feasibly result in a taxon which strongly resembles *I. hemivelata*.

Additional specimens examined:

COLOMBIA. **Departamentos Huila/Cauca:** Macizo Colombiano; páramo de Las Papas, Cerros y alrededores de la laguna La Magdalena, alt. 3530 m, 16 Oct 1958, *Idrobo, et al. 3307* (COL, NY). **Departamento Meta:** Páramo de Sumapaz, Hoya Sitiales, Laguna La Primavera y alrededores, alt. 3510 m, 9 Jan 1973, *Cleef 7555* (COL, U); Páramo de Sumapaz, Hoya El Nevado, Laguna La Guitarra y alrededores, alt. 3425 m, 23 Jan 1973, *Cleef 8268* (COL, U). **Departamento Risaralda:** Cordillera Central; Municipio de Pereira; parque de los Nevados, alt 4250 m, *Jaramillo, et al. 5700* (COL, U). **Departamento del Valle:** Páramo Pan de Azucar, alt 3300–3700 m, 23 Aug 1968, *Espinal & Ramos 2471* (COL).



### 3. *Isoëtes precocia* R.L. Small and Hickey, **sp. nov.** (Fig. 12–13, 18–19)

Type: "Venezuela: Anden, Estado Mérida: Sierra de St. Domingo, Páramo de Mucubaji, Weg zur Laguna Negra, an stark vernäßten Stellen,  $\pm$  3500 m. 4. 10. 1968." *B & F Oberwinkler 12968* (Holotype: M!; Isotype: VEN!).

*Isoëtes socia* sensu Fuchs-Eckert 1982 *pro parte*, Proc. Kon. Ned. Akad. Wetensch. C. 85(2): 256 1982. *non* A. Braun.

Species nova *I. karstenii* proxima, cujus megasporas laeves et velum completum habet; differt staturo minore, apicibus foliis brevissimis corneis et habitu ephemero.

Corm globose to horizontally elongate, two lobed, 1.8–2.4 mm ( $x = 2.1$ ) high, 2.5–4.6 mm ( $x = 3.6$ ) wide; dichotomously branching roots arising from the circumbasal fossa. Leaves 6–11, flexuous, to 63 mm long, to 4 mm wide at base, 0.5 to 0.9 mm wide at mid-length, leaf length / width ratio 45–157 ( $x = 80$ ); alae chartaceous, caramel-colored, extending 26–36% of total leaf length, apices acute to truncate; subula essentially terete in cross-section, pale green, apices blunt, corneous only at 0.1–0.2 mm of the tip; peripheral fibrous bundles, stomates, scale leaves and phyllopodia absent. Sporangia elliptic to circular, basal, unpigmented, 1.6–2.7 mm ( $x = 2.1$ ) long, 1.2–2.1 mm ( $x = 1.7$ ) wide. Velum complete, covering 100% of the sporangial surface (rarely slightly less). Ligule ephemeral, not seen. Labium absent or reduced to an extremely small transverse ridge of tissue on lower lip of the foveola. Megaspores white, lustrous, 388–495 ( $x = 437$ )  $\mu\text{m}$  in diameter, laevigate on both proximal and distal faces. Microspores cocoa brown, 27–30 ( $x = 29$ )  $\mu\text{m}$  in length 18.9–23.3 ( $x = 20.5$ )  $\mu\text{m}$  wide, echinate. Chromosome number:  $2n = 22$ .

*Isoëtes precocia* grows submerged in shallow, temporary pools at 3300–4400 m. It is currently known from Estado Mérida, Venezuela and Departamentos Boyaca, Cundinamarca and Meta in Colombia (Fig. 8). Although only eleven collections of this species have been found among the specimens examined, it is probably a common plant of seasonal pools but has not been collected due to its diminutive size and ephemeral nature. We visited the type locality in March of 1992, yet were unable to find this species because the pools were dry. Collections examined in this study were made in March, April, July, October, November and December, and all contained mega- and microspores.

Fuchs-Eckert (1982) recognized that a taxon resembling the type of *I. socia* existed, but failed to differentiate between the vegetative plantlets of *I. lechleri* in Peru and Bolivia and the distinct species (*I. precocia*) of Colombia and Venezuela. Hickey (1985) recognized that a distinct species existed, but did not study the associated specimens and based his acceptance of the name *I. socia* on the work of Fuchs-Eckert (1982). Hickey (1994) has subsequently placed *I. socia* in synonymy with *I. lechleri*.

Additional specimens examined:

COLOMBIA. **Departamento Boyacá:** Sierra Nevada del Cocuy, Alto Valle Lagunillas, 1.5 km NE de la Laguna Pintada, 4390 m, 30 Nov 1972, *Cleef 5704* (U); Sierra Nevada del Cocuy, Boqueron de Cusiri, alt. 4310 m, 5 Mar 1973, *Cleef 8785* (U); Páramo de la Sarna entre Sogamoso y Vado Hondo, alt. 3330 m., 8 Apr 1973, *Cleef 9522* (COL, U). **Departamento Cundinamarca:** Páramo de



Sumapaz, Chisaca, 100 m SW de la Laguna Larga, alt. 3700 m, 11 Dec 1971, *Cleef 153* (COL, U); Páramo de Cruz Verde, alt. 3200 m, 16 Oct 1961, *Idrobo & Hotheway 4174* (COL); Páramo de Sumapaz, vicinity of Laguna Chisaca, pool 50 m S of Laguna Larga, alt. 3620 m, 7 Jul 1986, *Keeley & Keeley 11032* (MU); Páramo de Sumapaz, vicinity of Laguna Chisaca, pool 50 m S of Laguna Larga, alt. 3620 m, 7 Jul 1986, *Keeley & Keeley 11033* (MU). **Departamento Meta:** Páramo de Sumapaz, Cerro Nevado del Sumapaz, alt. 3725 m, 21 Jan 1973, *Cleef 8185A* (COL).

VENEZUELA. **Estado Mérida:** Sierra de Sto. Domingo, Páramo de Mucubaji, alrededores de la Laguna Grande, alt. 3560–3600 m 19 Nov 1959, *Barclay & Juajibioy 9588* (COL, GH).

4a. ***Isoëtes karstenii*** A. Braun var. ***karstenii***. Verh. Bot. Vereins Prov. Brandenburg 3/4:332. 1862. Figs. 20–21.

Type: “Venezuela. Im Gebirge von Mérida 8000’ hoch in einem See ganz unter Wasser 1857.” *Karsten s.n.* (Holotype B!).

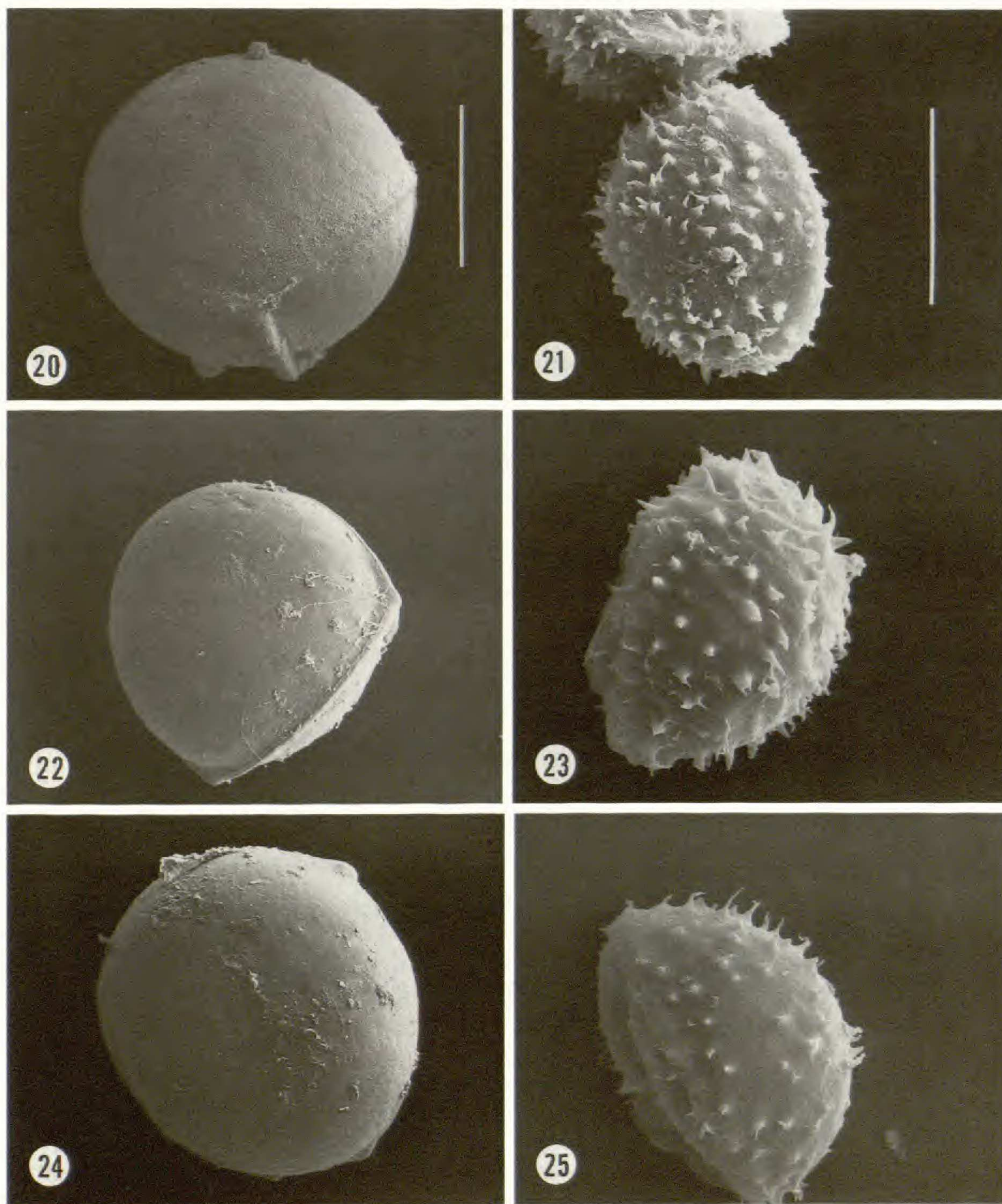
*Isoëtes lechleri* var. *colombiana* T. C. Palmer, Amer. Fern J. 19: 18. 1929. *I. colombiana* (T. C. Palmer) H. P. Fuchs, Proc. Kon. Ned. Akad. Wetensch. C. 85(2):259. 1982. Type: “Colombia, Dept. Santander: Laguna de Cunta, edge of Páramo de Santurban; alt. 3,880 m, 21 Jan 1927” *Killip & Smith 17964* (Holotype PH; Isotype: NY!), mixed collection.

*Isoëtes steyermarkii* Fuchs-Eckert, nom. nud. Proc. Koninkl. Ned. Acad. Wetensch. C85:257. 1982. Based on *Steyermark 55904* (US!).

Corm globose to horizontally elongate, two lobed (rarely 3), 2.0–4.0 mm ( $x = 2.8$ ) high, 2.8–7.8 mm ( $x = 5.2$ ) wide; dichotomously branching roots arising from the circumbasal fossa(e). Leaves 8–21, flexuous to stiffly erect, to 251 mm long, 5–10 mm wide at base, 0.9 to 2.4 mm wide at mid-length, leaf length/width ratio 35–157 ( $x = 77$ ); alae chartaceous, light-brown to blackish-brown, extending 14–38% of total leaf length, apices acute to truncate; subula essentially terete in cross-section, olive green, apices blunt, often spatulate, distal 0.5–3.2 mm of apex corneous; peripheral fibrous bundles, stomates, scale leaves and phyllopodia absent. Sporangia widely elliptic to circular, basal, unpigmented, 2.1–5.5 ( $x = 3.4$ ) mm long, 1.8–3.9 ( $x = 2.6$ ) mm wide. Velum complete, covering 100% of the sporangial surface (rarely slightly less). Ligule deltate, auriculate, 0.5–1.5 mm high, 0.75–2.0 mm wide. Labium absent or reduced to an extremely small transverse ridge on lower lip of the foveola. Megaspores white, lustrous, 400–507 ( $x = 467$ )  $\mu\text{m}$  in diameter, laevigate on both proximal and distal faces. Microspores cocoa brown, 25–38 ( $x = 30$ )  $\mu\text{m}$  in length, 17.7–28.7 ( $x = 20.8$ )  $\mu\text{m}$  wide, echinate. Chromosome number:  $2n = 22$ .

*Isoëtes karstenii* grows submerged in permanent lakes and ponds (occasionally streams) between 3300–4600 m. It is distributed throughout the eastern cordillera of the northern Andes from Estado Mérida, Venezuela, to Departamentos Arauca, Boyaca, Cundinamarca, Meta, Tolima and Nariño in Colombia (Fig. 8). The collections of this species were made from every month except August and October. With the exception of the single collection made in June,





FIGS. 20–25. *Isoëtes karstenii* var. *karstenii*, *I. karstenii* var. *anomala*, and *I. palmeri*. FIGS. 20–21—SEM of spores of *Isoëtes karstenii* var. *karstenii*. FIG. 20. Near equatorial view of megaspore (Cleef 8599, BM). Bar = 200  $\mu$ m. FIG. 21. Equatorial view of microspore (Oberwinkler & Oberwinkler 13685, M). Bar = 20  $\mu$ m. FIGS. 22–23—SEM of spores of *I. karstenii* var. *anomala* (Rimbach 171, US). FIG. 22. Distal view of megaspore. Scale as in Fig. 20. FIG. 23. Equatorial view of microspore. Scale as in Fig. 23. FIGS. 24–25—SEM of spores of *I. palmeri* (Cleef 8647, BM). FIG. 24. Distal view of megaspore. Scale as in Fig. 20. FIG. 25. Equatorial view of microspore. Scale as in Fig. 21.



specimens from all other months contained both mega- and microspores, suggesting this species is evergreen and fertile year-round. This species is the most widely distributed (or at least most widely collected) member of this group.

*Isoëtes colombiana* (T. C. Palmer) H. P. Fuchs and *I. steyermarkii* H. P. Fuchs nom. nud. are synonyms of *I. karstenii*. Palmer (1929) originally separated *I. lechleri* var. *colombiana* from *I. lechleri* var. *lechleri* on the basis of the absence of an equatorial ridge on the megaspores, the small size of the microspores, and the corneous leaf apices. All of these characters agree with the current circumscription of *I. karstenii*. In addition, the type collection of *I. lechleri* var. *colombiana* is a mixture of fertile *I. karstenii* and plants of apparent hybrid origin. The hybrids are the specimens that contain ornamented and misshapen megaspores as noted in Palmer's (1929) protologue. *Isoëtes steyermarkii* was invalidly published by Fuchs-Eckert (1982) and no description was given. Examination of the basis for this taxon (*Steyermark 55904*) reveals no characters that differentiate it from *I. karstenii*; in fact this collection is nearly identical to the type of *I. karstenii*.

Finally, the type specimen of *I. karstenii* as cited by Braun (1862) is "In einem ungefahr 8000' hoch See auf dem Páramo im Gebirge von Mérida (Venezuela) ganz unter Wasser im Jahre 1853 von Prof. H. Karsten entdeckt, . . ." The specimen at Berlin which was annotated as the holotype by H. P. Fuchs bears the label "*Isoëtes karstenii* A. Braun. Venezuela. Im Gebirge von Mérida 8000' hoch in einem See ganz unter Wasser. C. Dr. Karsten etc. 1857." Although the locality information on this label closely matches that of Braun's (1862) description, there is a discrepancy regarding the date of collection: 1853 in the description and 1857 on the specimen label. Stafleu and Cowan (1979) indicate that Karsten was in South America from 1848 to 1856, but returned to Berlin in 1856 where he remained until 1868. Thus, he could not have collected this specimen in 1857. A potential explanation for this discrepancy could be that 1853 was the date of collection, while 1857 was the date the material was accessioned into the Berlin herbarium.

Additional specimens examined:

**COLOMBIA. Departamento Arauca:** Cordillera Oriental, extremo sur de la Sierra Nevada del Cocuy, Laguna el Amarillal, alt. 4065 m, 23 Mar 1977, *Cleef & van der Hammen 10383* (U); Sierra Nevada del Cocuy, Laguna de la Plaza, alt. 4550 m, 20–30 Jan 1959, *van der Hammen & Gonzalez 1374* (COL); Sierra Nevada del Cocuy, Laguna la Plaza, alt. 4150 m, Mar 1977, *van der Hammen 4753* (COL, U). **Departamento Boyacá:** Sierra Nevada del Cocuy, Alto Valle Lagunillas, Laguna Cuadrada, alt. 4060 m, 26 Sept. 1972, *Cleef & Florschütz 5590* (COL, U); Sierra Nevada del Cocuy, Páramo Concavo, 3 km al N del morro Pulpito del Diable, alt. 4410 m, 27 Feb 1973, *Cleef 8599* (U); Sierra Nevada del Cocuy, Alto Valle Lagunillas, medio km al WNW de la Laguna Pintada, alt. 4000 m, 4 Mar 1973, *Cleef 8782* (COL, U); Municipio de El Cocuy, valle del rio Lagunillas, Sierra Nevada del Cocuy, Laguna La Pintada, alt. 3800 m, 23 May 1993, *Small, Gonzalez & Ruiz 141–143* (COL, MU); Municipio de El Cocuy, valle del rio Lagunillas, Sierra Nevada del Cocuy, Laguna La Cuadrada, alt. 3900 m, 23 May 1993, *Small, Gonzalez & Ruiz 144* (COL, MU). **Departamento Cundinamarca:** Páramo de Sumapaz, Chisaca, Laguna Negra, alt. 3750 m, 11 Dec 1971, *Cleef 174* (COL, U); Km 31, south of Usme, Laguna Chisaca, alt. 3620 m, 3 Jan 1985, *Keeley & Keeley 7908* (COL, MU); Km 31, south of Usme, Laguna Chisaca, alt. 3620 m, 3 Jan 1985, *Keeley & Keeley 7909* (COL, MU); Small laguna adjacent to north side of road at km 31 between Laguna Chisaca and Laguna Larga, alt. 3650 m, 10 Dec 1985, *Keeley & Keeley 10076* (MO, MU); Unnamed laguna on



opposite side of road from largest laguna at km 30.5, Páramo de Sumapaz, vicinity of Laguna Chisaca, alt. 3650 m, 10 Dec 1985, *Keeley & Keeley 10082* (MO, MU); Páramo de Sumapaz, laguna at km 30.5 opposite side of road from Laguna Chisaca, alt. 3700 m, 15 Dec 1985, *Keeley & Keeley 10093* (MU); Páramo de Sumapaz, vicinity of Laguna Chisaca, laguna adjacent to road at km 31, alt. 3620 m, 7 Jul 1986, *Keeley & Keeley 11037* (MU); Páramo, alt. 3700 m, Apr 1963, *Saravia 2533* (COL). **Departamento Meta:** Páramo de Sumapaz, Laguna La Guitarra, alt. 3460 m, 21 Jan 1972, *Cleef 832* (COL, U); Páramo de Sumapaz, Laguna La Primavera, alt. 3525 m, 25 Jan 1972, *Cleef 1017* (COL, U); Páramo de Sumapaz, Cerro Nevado del Sumapaz, alt. 4050 m, 30 Jan 1972, *Cleef 1351* (COL, U); Páramo de Sumapaz, Cerro Nevado del Sumapaz, alt. 4090 m, 13 Jan 1973, *Cleef 7766* (COL, U); Páramo de Sumapaz, Laguna Los Sitiales, alt. 3620 m, 22 Jan 1973, *Cleef 8248* (COL, U); Macizo de Sumapaz, Boqueron del Palacio, norte de Cerro del Nevado, alt. 3900 m, 5 Jul 1981, *Diaz, et al. 2558* (COL); Macizo de Sumapaz, Laguna La Guitarra, alt. 3370 m, 2 Jun 1981, *Diaz & Rangel 2390* (COL). **Departamento Nariño:** Cumbal, Laguna La Bolsa, alt. 3400 m, 21 Jan 1973, *Hagemann & Leist 1968* (COL); Laguna Negra en vecindades de Pasto, alt. 3520 m, 17 Jul 1973, *Leist & Möhle 2212* (COL).

VENEZUELA. **Estado Mérida:** Laguna Grande de Mucubaji, alt. 3535 m, 23 Dec 1984, *Keeley & Keeley 7861* (MU); Laguna Grande de Mucubaji, alt. 3535 m, 23 Dec 1984, *Keeley & Keeley 7862* (MU); Laguna Negra, alt. 3430 m, 23 Dec 1984, *Keeley & Keeley 7865* (MU); Laguna de los Patos, alt. 3670 m, 23 Dec 1984, *Keeley & Keeley 7868* (MU); Laguna Negra, alt. 3400 m, 22 Nov. 1968, *Oberwinkler & Oberwinkler 13685* (M); Laguna St. Barbara, alt. 3700 m, 11 Jan 1969, *Oberwinkler & Oberwinkler 14272* (M); Laguna St. Barbara, alt. 3700 m, 11 Jan 1969, *Oberwinkler & Oberwinkler 14274* (M); Small pond ca. 8 km N of Pico de Aguila on road to Pinango, alt. 3500 m, 15 Mar 1992, *Small & Hickey 4* (MU); Small pond ca. 15 km N of Pico de Aguila on road to Pinango, alt. 3500 m, 15 Mar 1992, *Small & Hickey 5* (MU); Small pond ca. 15 km N of Pico de Aguila on road to Pinango, alt. 3500 m, 15 Mar 1992, *Small & Hickey 6* (MU); Laguna Negra, alt. 3500 m, 17 Mar 1992, *Small & Hickey 7* (MU); Laguna Mucubaji, alt. 3500 m, 17 Mar 1992, *Small & Hickey 8* (MU); Near upper limits of páramo, around small lake between Chachapo and Los Apartaderos, near El Aguila, 3930 m, 15 Apr 1944, *Steyermark 55904* (F, US, VEN); Laguna Grande de Mucubaji, alt. 3500 m 19 Sep 1961, *Tryon & Tryon 5839* (GH).

**4b. *Isoëtes karstenii* var. *anomala* (T. C. Palmer) R.L. Small and Hickey, comb. nov.** Figs. 22–23.

*Isoëtes lechleri* var. *anomala* T. C. Palmer, Amer. Fern J. 22: 130–131. 1932. *Isoëtes rimbachiana* H. P. Fuchs Proc. Kon. Ned. Akad. Wetensch. C. 85(2): 259. 1982. Type: “Ecuador. Mt. Chimborazo. Submerged in water of shallow pond. Rooting in mud. 4200 m.” *Rimbach 171* (Holotype US!)

*Isoëtes karstenii* var. *anomala* differs from *I. karstenii* var. *karstenii* only in the character of velum coverage: 25–75% in var. *anomala*, >90% in var. *karstenii*. In Palmer’s (1932) description of this taxon (as *I. lechleri* var. *anomala*) he distinguishes it from other “*I. lechleri*” (of which he considered *I. karstenii* to be a synonym) based primarily on the presence of a two-layered velum which incompletely covered the sporangium. Examination of the holotype, however, revealed no indication of a two-layered velum on the sporophylls we observed. The presence of a “multilayered velum” has been reported elsewhere (e.g., *Isoëtes prototypus*, Britton and Goltz, 1991). Initial investigations indicate that the apparent multilayered velum is actually due to disintegration of the mesophyll of the velum and separation of the upper and lower epidermal layers resulting in what appears as a double velum (Hickey, unpublished data). Regardless, this taxon appears unique in the extent of velum coverage.



It is otherwise nearly identical to the type of *I. karstenii*, as noted by Palmer (1932). This taxon is known only from the type locality (Mt. Chimborazo, Ecuador) although Rodriguez (1955) cites one specimen from Laguna Negra, Mérida, Venezuela as *I. lechleri* var. *anomala*.

5. ***Isoëtes palmeri*** H. P. Fuchs. Proc. Kon. Ned. Akad. Wetensch. C. 84 (2): 168–173. 1981. Figs. 24–25.

Type: "Venezuela; Laguna Grande de Apartaderos, Mérida, floating on water, 21 January 1929" Pittier 13242 (Holotype US!; Isotypes G!, M!, MO!, VEN!).

*Isoëtes cleefii* H. P. Fuchs Proc. Kon. Ned. Akad. Wetensch. C. 84 (2):177–181. 1981. Type: "Colombia, Cundinamarca: Páramo de Sumapaz, páramo y bosque alto-andino cerca de Lagunitas al S. de San Juan. Laguna Gobernador: orilla W." Cleef 8308 (Holotype COL!; Isotype COL!).

Corm globose to horizontally elongate, two lobed, 1.3–6.0 mm ( $x = 2.9$ ) high, 3.0–8.5 mm ( $x = 5.0$ ) wide; dichotomously branching roots arising from the circumbasal fossa. Leaves 5–25, flexuous, to 350 mm long, 5–8 mm wide at base, 0.5 to 1.5 mm wide at mid-length, leaf length/width ratio 118–350 ( $x = 241$ ); alae chartaceous, dark brown to blackened distally, pale proximally where leaf was imbedded in substrate, extending 10–27% of total leaf length, apices acute to truncate; subula essentially terete in cross-section, pale green, apices blunt, often spatulate, distal 1.0–2.5 mm of apex sclerified and blackened; peripheral fibrous bundles, stomates, scale leaves and phyllopodia absent. Sporangia elliptic to obovate, basal, unpigmented, 2.5–5.9 ( $x = 3.8$ ) mm long, 1.9–4.0 ( $x = 2.8$ ) mm wide. Velum complete, covering 100% of the sporangial surface (rarely slightly less). Ligule deltate, auriculate, to 1 mm high, to 1 mm wide. Labium absent or reduced to an extremely small transverse ridge on lower lip of the foveola. Megaspores white, lustrous, 380–550 ( $x = 460$ )  $\mu\text{m}$  in diameter, laevigate on both proximal and distal faces. Microspores cocoa brown, 26–36 ( $x = 30.4$ )  $\mu\text{m}$  in length, 17.9–26.4 ( $x = 21.3$ )  $\mu\text{m}$  wide, echinate. Chromosome number:  $2n = 44$ .

*Isoëtes palmeri* grows submerged in permanent pools, ponds, and lakes between 3340–4435 m. It is distributed in the eastern cordillera of the northern Andes from Estado Mérida, Venezuela, through the Departamentos Arauca, Boyaca, Cundinamarca and Meta of Colombia (Fig. 8).

The presence of both microspores and megaspores in all months for which collections are available (nine out of twelve) suggests that this species is evergreen and fertile year-round.

*Isoëtes palmeri*'s affiliation with the *I. karstenii* complex is apparent by its laevigate megaspores, acute to truncate alae apices, and corneous leaf tips. For the most part, *I. palmeri* and *I. karstenii* are easily differentiated morphologically; *I. palmeri* has long, slender leaves which appear flexuous, and *I. karstenii* has shorter, wider leaves which usually appear rigid and stiff. Occasional collections appear intermediate between these two extremes. These col-



lections often represent juvenile individuals of either species, but may also include hybrids.

*Isoëtes cleefii* is a synonym of *I. palmeri*. Although the holotype and one of the paratypes of *I. cleefii* have leaves that are slightly longer and wider than typical *I. palmeri*, these individuals are merely extreme variants within a wide range of intraspecific variation. Both *I. palmeri* and *I. cleefii* were published by Fuchs-Eckert (1981a,b) at the same time, thus neither name has priority. We select the name *I. palmeri* to represent this species because the type collection is more representative of the species.

Additional specimens examined:

COLOMBIA. **Departamento Arauca:** Sierra Nevada del Cocuy, Cabeceras de la Quebrada El Playon, alt. 4245 m, 11 Mar 1973, *Cleef* 9088 (COL, U). **Departamento Boyacá:** Páramos al NW de Belen, ca. 600 m NE de la Laguna el Alcohol, alt. 3850 m, 29 Feb 1972, *Cleef* 2059 (COL, U); Páramo de la Rusia, NW-N de Duitama, alt. 3570 m, 13, Dec 1972, *Cleef* 7144 (COL, U); Sierra Nevada del Cocuy, Páramo Concavo, alt. 4435 m, 28 Feb 1973, *Cleef* 8647 (COL, U); Páramo de la Rusia, NW-N de Duitama, Laguna Negra, alt. 3745 m, 14 Dec 1972, *Cleef* 7221 (COL, U); Sierra Nevada del Cocuy, Páramo Concavo, 2.5 km al N de morro Pulpito del Diablo, alt. 4435 m, 28 Feb 1973, *Cleef* 8648 (COL, U); Páramo entre Peña Arnical y Alto de Mogotes; lagunita 0.5 km al SE de la Laguna Grande, alt. 3340 m, 1 Apr 1973, *Cleef* 9273 (COL, U); Páramo entre Pena de Arnical y Alto de Mogotes, cerca de la Laguna Grande, alt. 3350 m, 10 Apr 1973, *Cleef* 9551 (COL, U); Páramos al NW de Belen, vereda San Jose de la Montana, alt. 3725 m, 3 May 1973, *Cleef* 9699 (U); Páramo de Pisva, carretera Socha-La Punta km 77, Laguna Colorada, alt. 3425 m, 22 May 1973, *Cleef* 9898 (COL, U); Largest laguna, 17 km NW of Belen on road to San Jose de Montana, alt. 3700 m, 1 Jan 1985, *Keeley & Keeley* 7887 (COL, MU). **Departamento Cundinamarca:** Páramo entre Cogua y San Cayetano, Laguna Verde, 3600 m, 30 Nov 1971, *Cleef* 80 (COL, U); Páramo de Sumapaz, hoyo la Laguna Larga, alt. 3750 m, 14 Dec 1971, *Cleef & Jamarillo* 262 (COL, U); Páramo de Palacio, Lagunas Buitrago, alt. 3600 m, 16 Dec 1971, *Cleef* 296 (COL, U); Páramo de Sumapaz, Andabobos, alt. 3800 m, 9 Feb 1972, *Cleef* 1553 (COL, U); Páramo de Palacio, Lagunas de Buitrago, alt. 3580 m, 25 May 1972, *Cleef* 4112 (COL, U); Páramo de Sumapaz, lagunitas al S de San Juan, Laguna Gobernador, alt. 3815 m, 26 Jan 1973, *Cleef* 8308 (COL); Páramo de Chingaza, cerca de la "Paila", 10–20 Jan 1965, *Huertas & Camargo* 6025 (COL); Cordillera Oriental, Macizo de Sumapaz, Laguna de Chisaca, alt. 3680 m, 14 Apr 1958, *Idrobo* 2741 (COL); Laguna Seca, N of Zipaquire, alt. 3700 m, 30 Dec 1984, *Keeley & Keeley* 7876 (MU); Laguna Seca, N of Zipaquire, alt. 3700 m, 30 Dec 1984, *Keeley & Keeley* 7878 (COL, MU); Páramo de Sumapaz, vicinity of Laguna Chisaca, Laguna Larga, alt. 3650 m, 10 Dec 1985, *Keeley & Keeley* 10072 (MO, MU); Páramo de Sumapaz, vicinity of Laguna Chisaca, Laguna Larga, alt. 3650 m, 10 Dec 1985, *Keeley & Keeley* 10073 (MO, MU); Páramo de Sumapaz, vicinity of Laguna Chisaca, small pools S of Laguna Larga, alt. 3650 m, 10 Dec 1985, *Keeley & Keeley* 10077 (MO, MU); Páramo de Sumapaz, vicinity of Laguna Chisaca, small pools ca. 100 m above SE end of Laguna Chisaca, alt. 3700 m, 13 Dec 1985, *Keeley & Keeley* 10088 (MO, MU); Páramo de Sumapaz, Laguna Chisaca, alt. 3700 m, 13 Dec 1985, *Keeley & Keeley* 10090 (MU); Páramo de Sumapaz, vicinity of Laguna Chisaca, small pool ca. 200 m above SE end of laguna on NE side of road at km 30.5, alt. 3700 m, 13 Dec 1985, *Keeley & Keeley* 10091 (MU); Páramo de Sumapaz, vicinity of Laguna Chisaca, laguna ca 100 m NE of km 31 lagunita, alt. 3700 m, 13 Dec 1985, *Keeley & Keeley* 10092 (MO, MU); Páramo de Sumapaz, vicinity of Laguna Chisaca, Laguna Larga, alt. 3620 m, 7 Jul 1986, *Keeley & Keeley* 11029 (MU); Páramo de Sumapaz, vicinity of Laguna Chisaca, shallow pool on ridge SE of first lake, alt. 3620 m, 7 Jul 1986, *Keeley & Keeley* 11039 (MU); Carretera Cogua-San Cayetano, Páramo de Laguna Seca, alt. 3660 m, 3 Aug 1970, *Piedrahita* 276 (COL). **Departamento Meta:** Páramo de Sumapaz, Laguna la Primavera, alt. 3510 m, 9 Jan 1973, *Cleef* 7556 (COL, U); Páramo de Sumapaz, Lagunas el Sorbedero y El Nevado, alt. 3635 m, 16 Jan 1973, *Cleef* 7910 (COL, U); Páramo de Sumapaz, Laguna la Guitarra, alt. 3425 m, 23 Jan 1973, *Cleef* 8267 (COL).



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